The Use of a Selenium-Peptide to Specifically Inactivate *Yersinia pestis*

Report Documentation Page

Report Documentation Page					*Form Approved* *OMB No. 0704-0188*

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1. REPORT DATE **01 OCT 2005**	2. REPORT TYPE **N/A**	3. DATES COVERED **-**
4. TITLE AND SUBTITLE **The Use of a Selenium-Peptide to Specifically Inactivate Yersinia pestis**		5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)		5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Texas Tech University Health Sciences Center**		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S) 11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003. , The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**UU**	**102**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

The Use of a Selenium-Peptide to Specifically Inactivate *Yersinia pestis*

Joe A. Fralick, Ph.D.

The Use of a Selenium-Peptide to Specifically Inactivate *Yersinia pestis*

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Phat Tran, B.A.

Overview of project

- Develop an antibiotic that will selectively kill Y. pestis without killing other bacteria

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- Develop an antibiotic that will selectively kill Y. pestis without killing other bacteria
- Use a killing mechanism for which the bacteria can not develop a resistance

Selectivity:

a. We need a targeting mechanism to bind to a specific protein from Y. pestis.

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Selectivity:

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b. The F1 protein on the surface of Y. pestis is known to function as a good vaccine for the bacteria.

c. Peptides are known to be very selective in their binding.

d. Peptides are known to have high affinity binding.

e. Peptides are less expensive to produce, more stable and are easier to deliver to a target in vivo than antibodies.

Experimental Approach:

a. Find a peptide that will bind selectively to the F1 protein with high affinity.

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b. Libraries of peptides are available which contain large numbers (10^9) of different peptides.

c. We need to isolate a single peptide from the library that will bind to the F1 antigen.

The problem:

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The Solution:

PHAGE DISPLAY!

The power of phage display lies in the fact that it creates a physical linkage between a selectable function (the displayed peptide sequence) and the DNA encoding that function.

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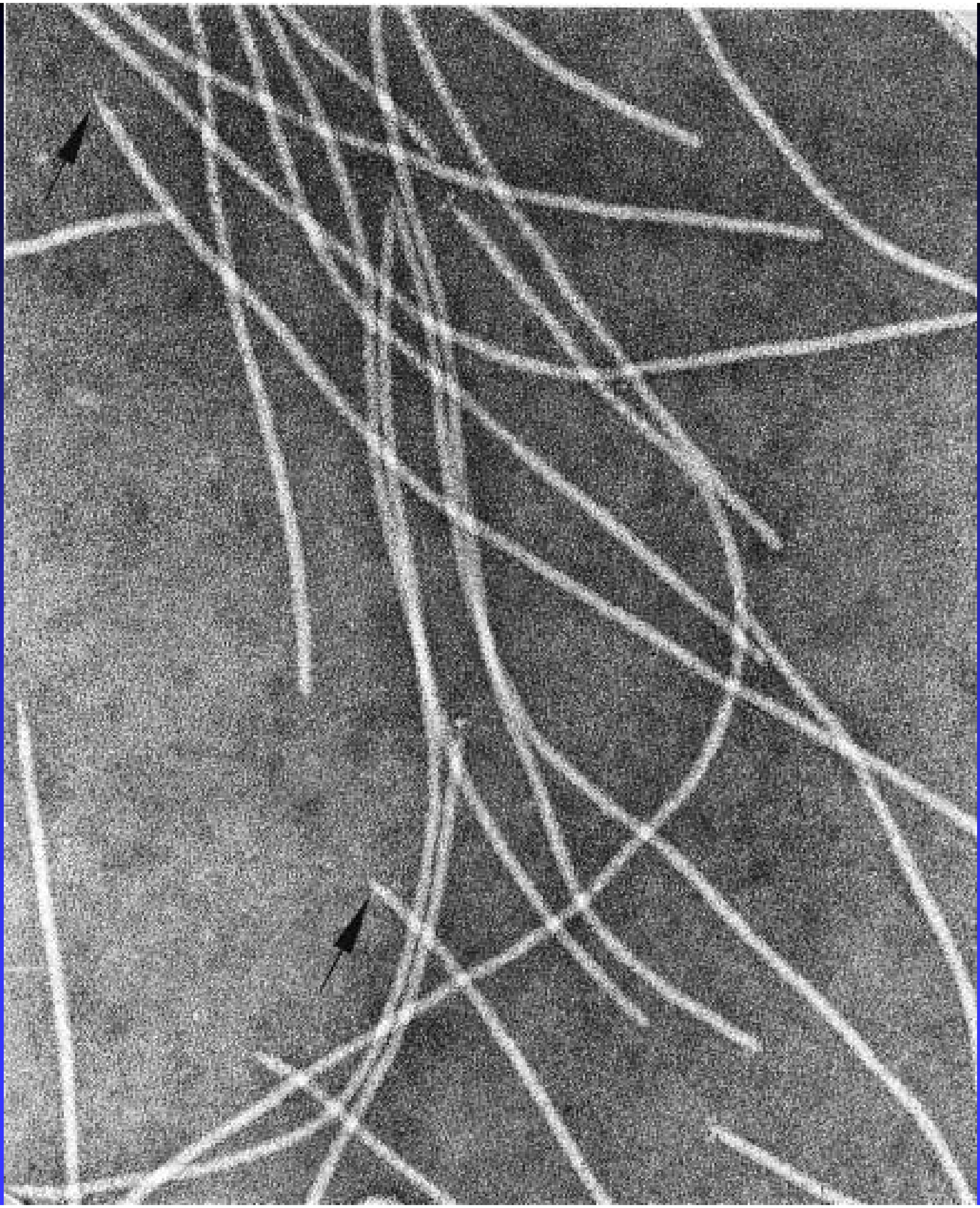
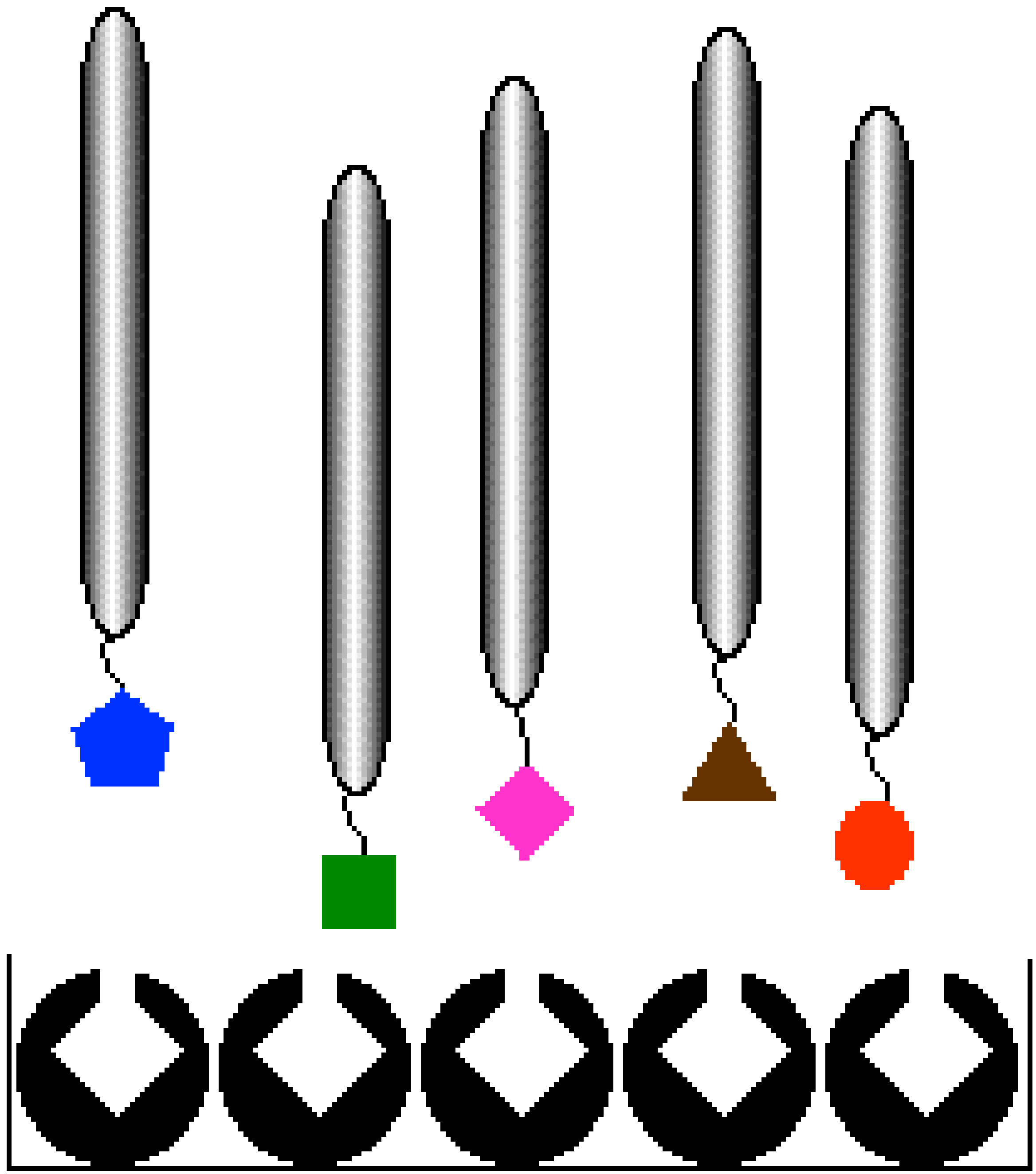
The power of phage display lies in the fact that it creates a physical linkage between a selectable function (the displayed peptide sequence) and the DNA encoding that function.

In other words, the peptide comes with its own built in message that tells us its sequence. A message that we can determine from a single isolated phage.



Filamentous Bacteriophage

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.



Affinity Selection or Biopanning:

10^{11} phage + target

Wash off unbound phage

Harvest bound phage

Amplify selected phage

Sequence Phage DNA

Stringency

The stringency of affinity selection is controllable in some degree by the choice of conditions used in biopanning experiments.

Low Stringency

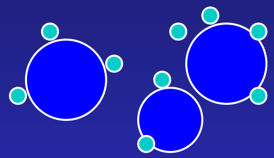
Presence of detergents, soluble vs fixed target, temperature, competition agents, etc.

High Stringency

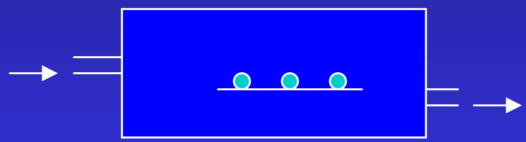
Biopanning:

Microtiter plates

Beads



BiaCore

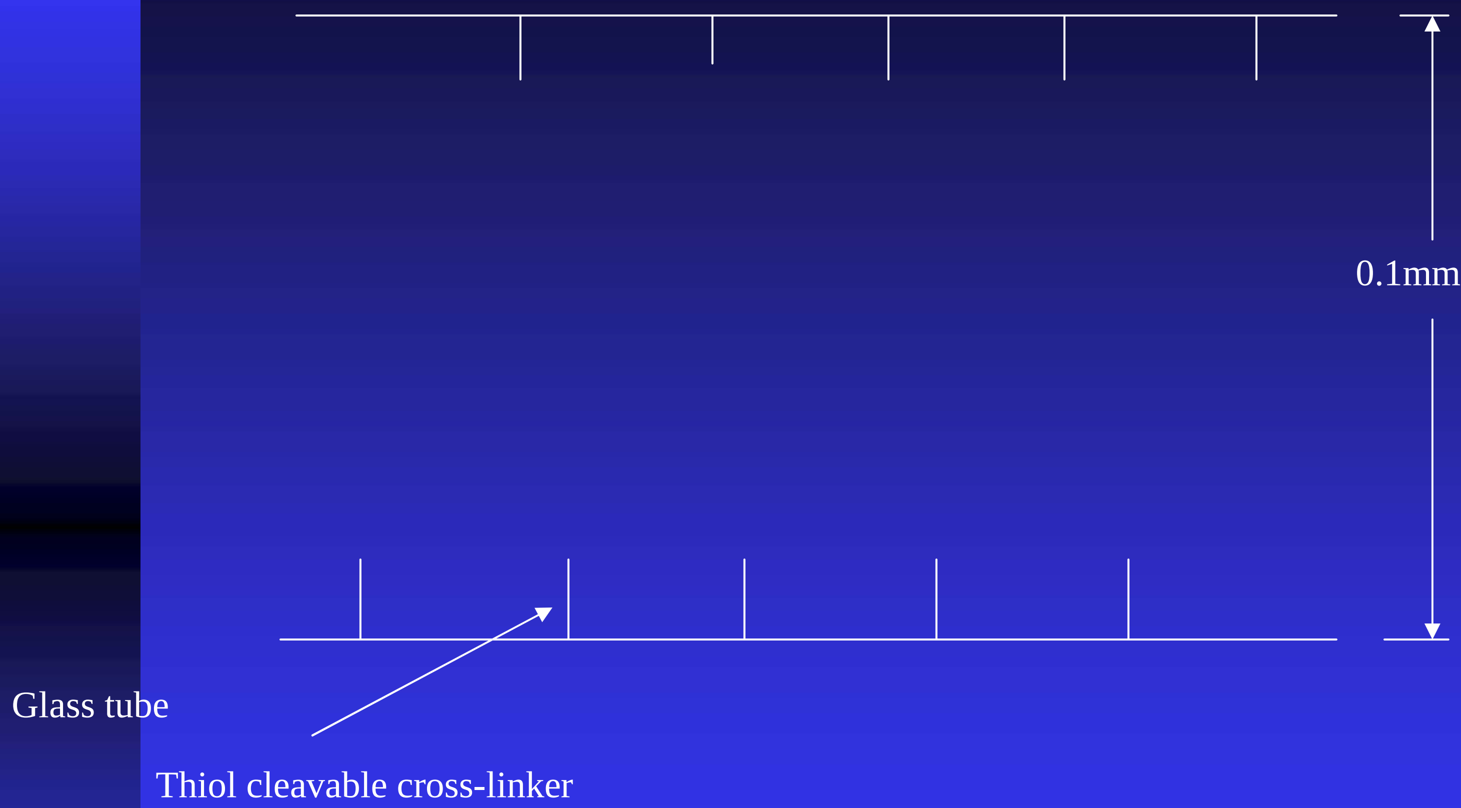


>$100,000

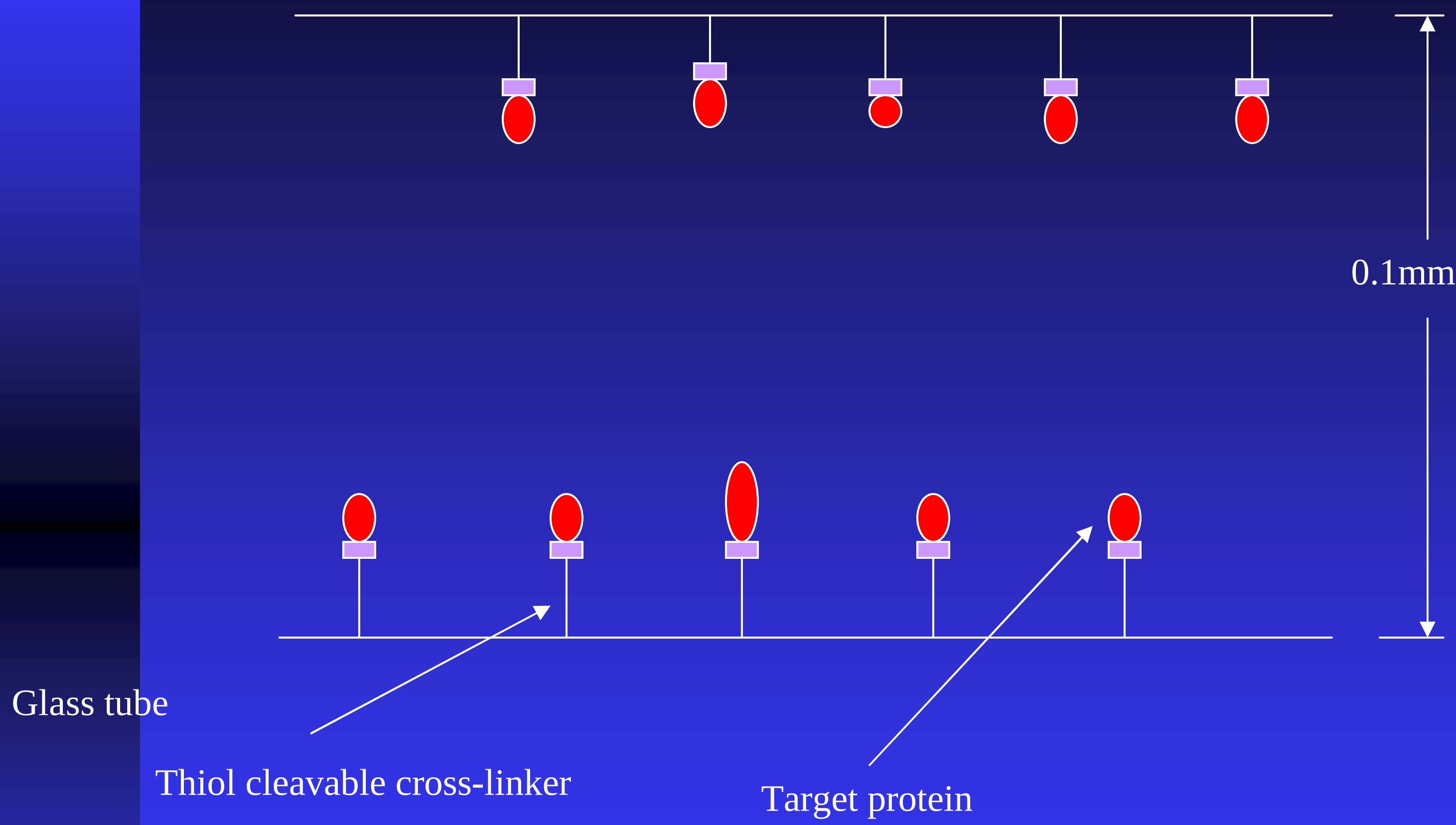
Glass tube biopanning apparatus

Glass tube

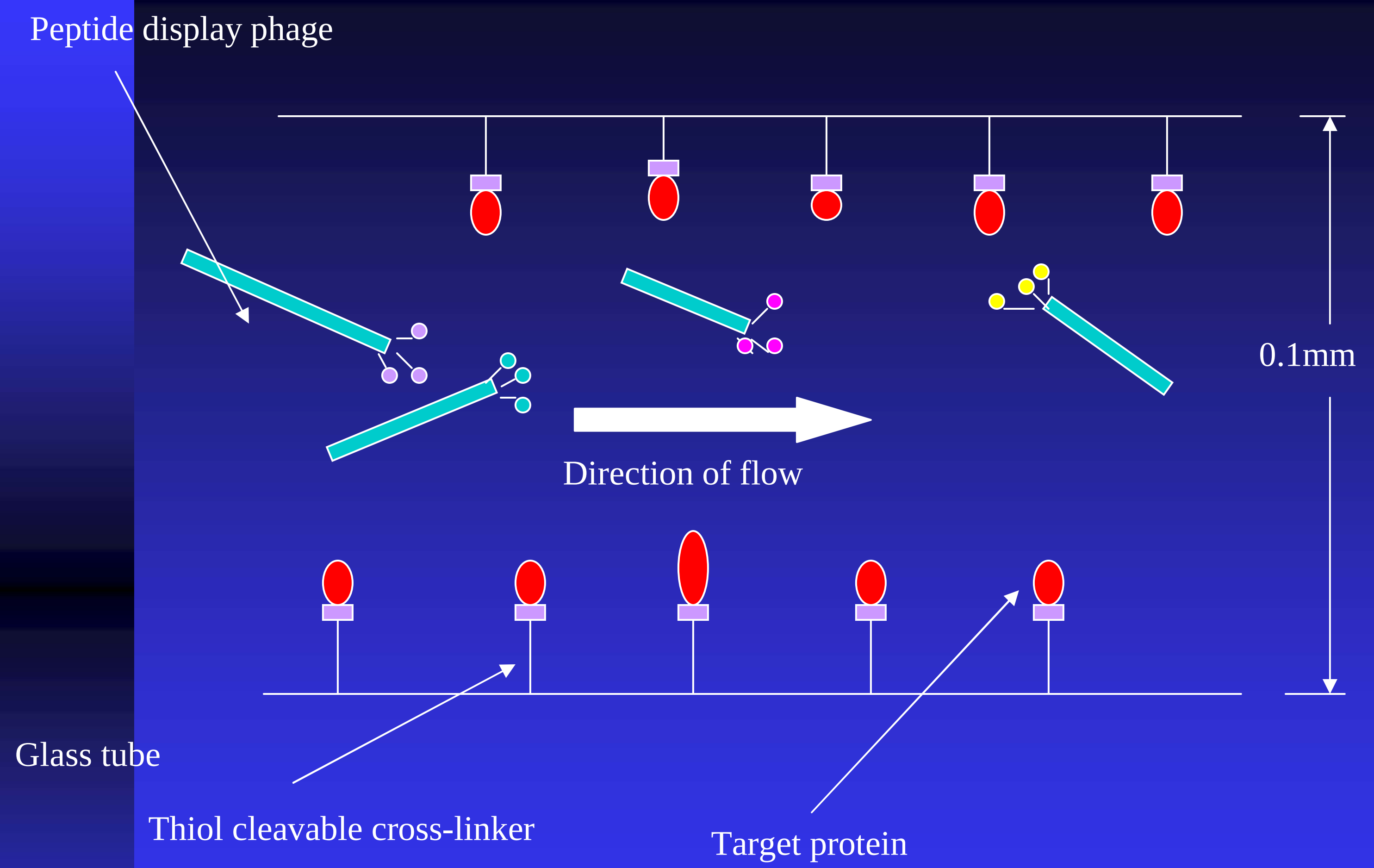
Glass tube biopanning apparatus



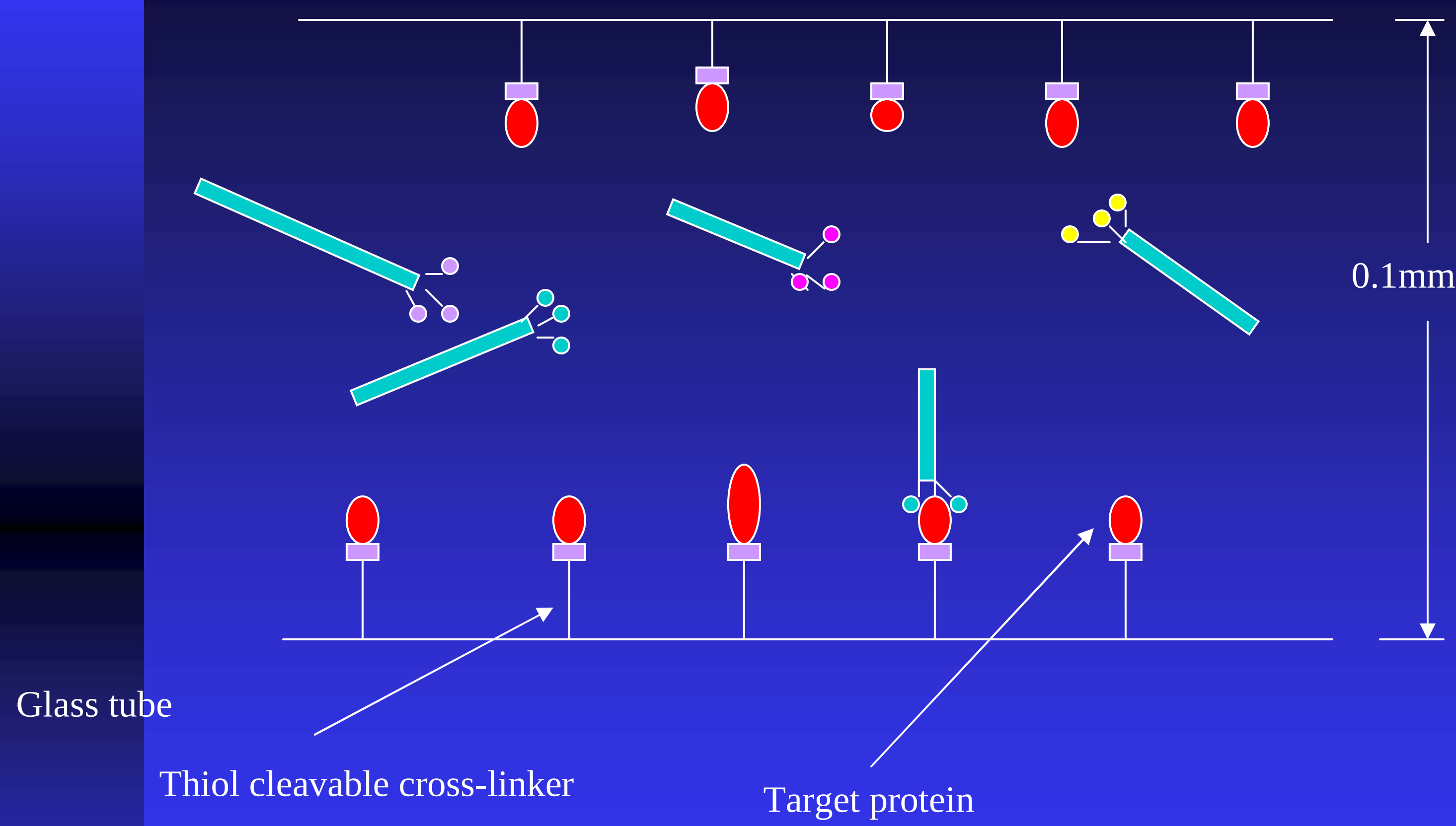
Glass tube biopanning apparatus



Glass tube biopanning apparatus



Glass tube biopanning apparatus



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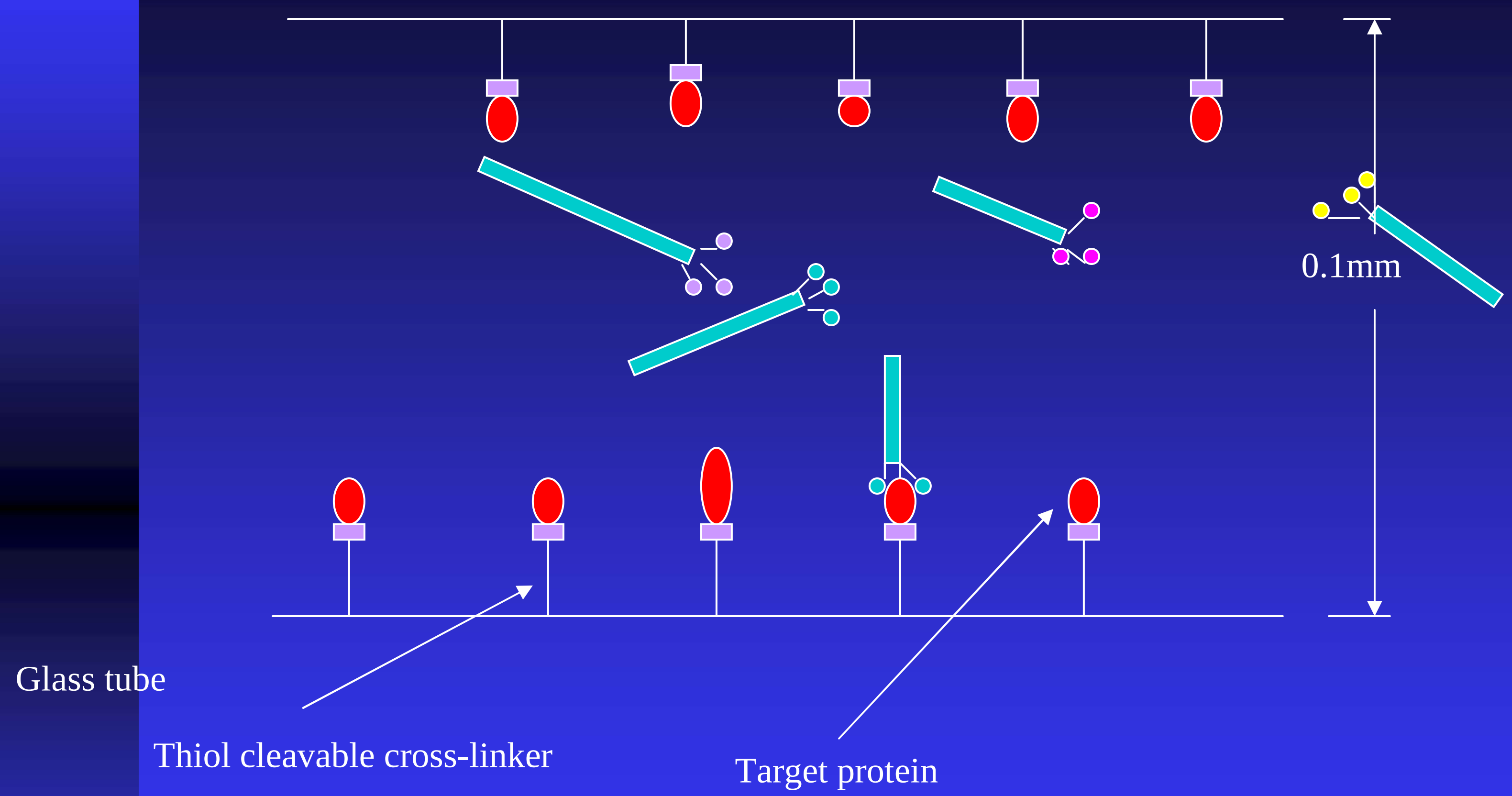

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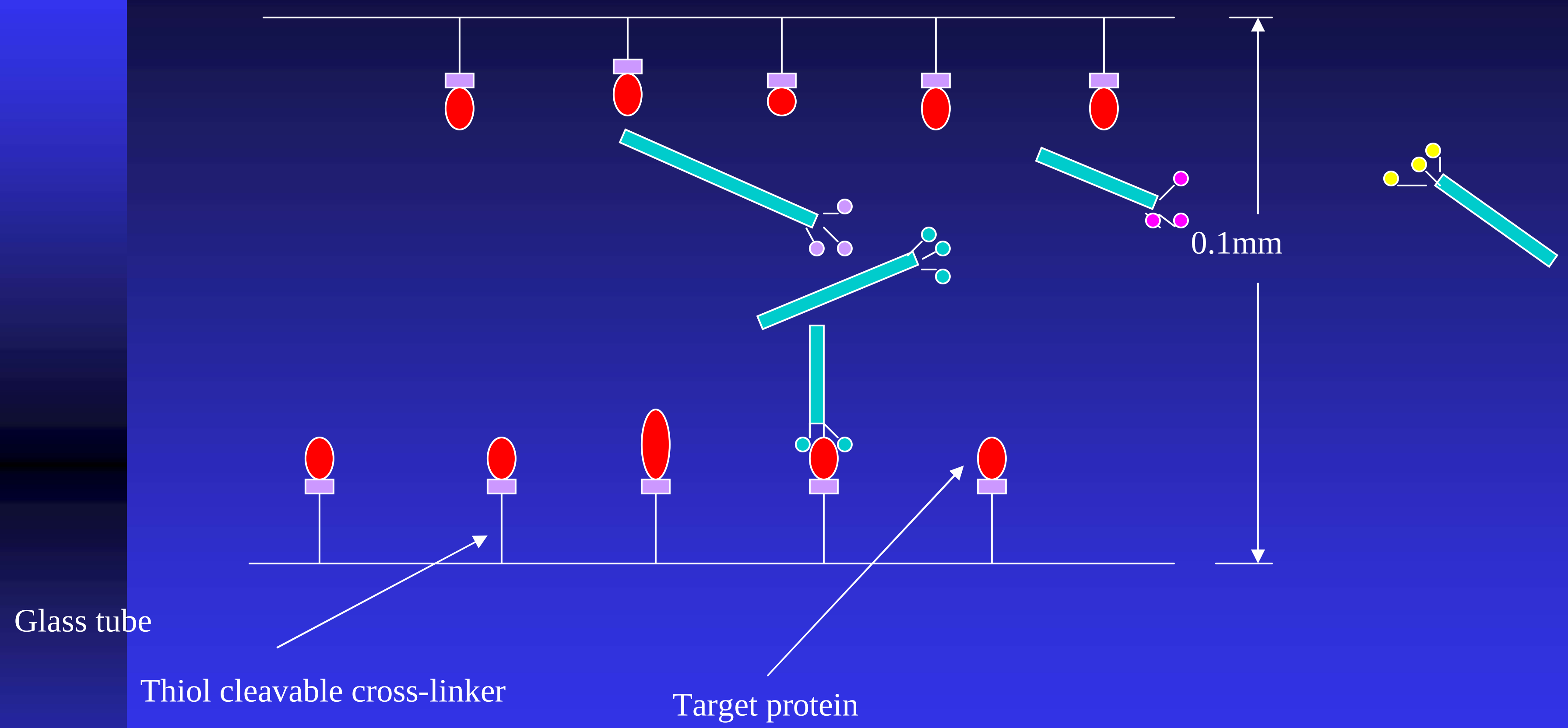

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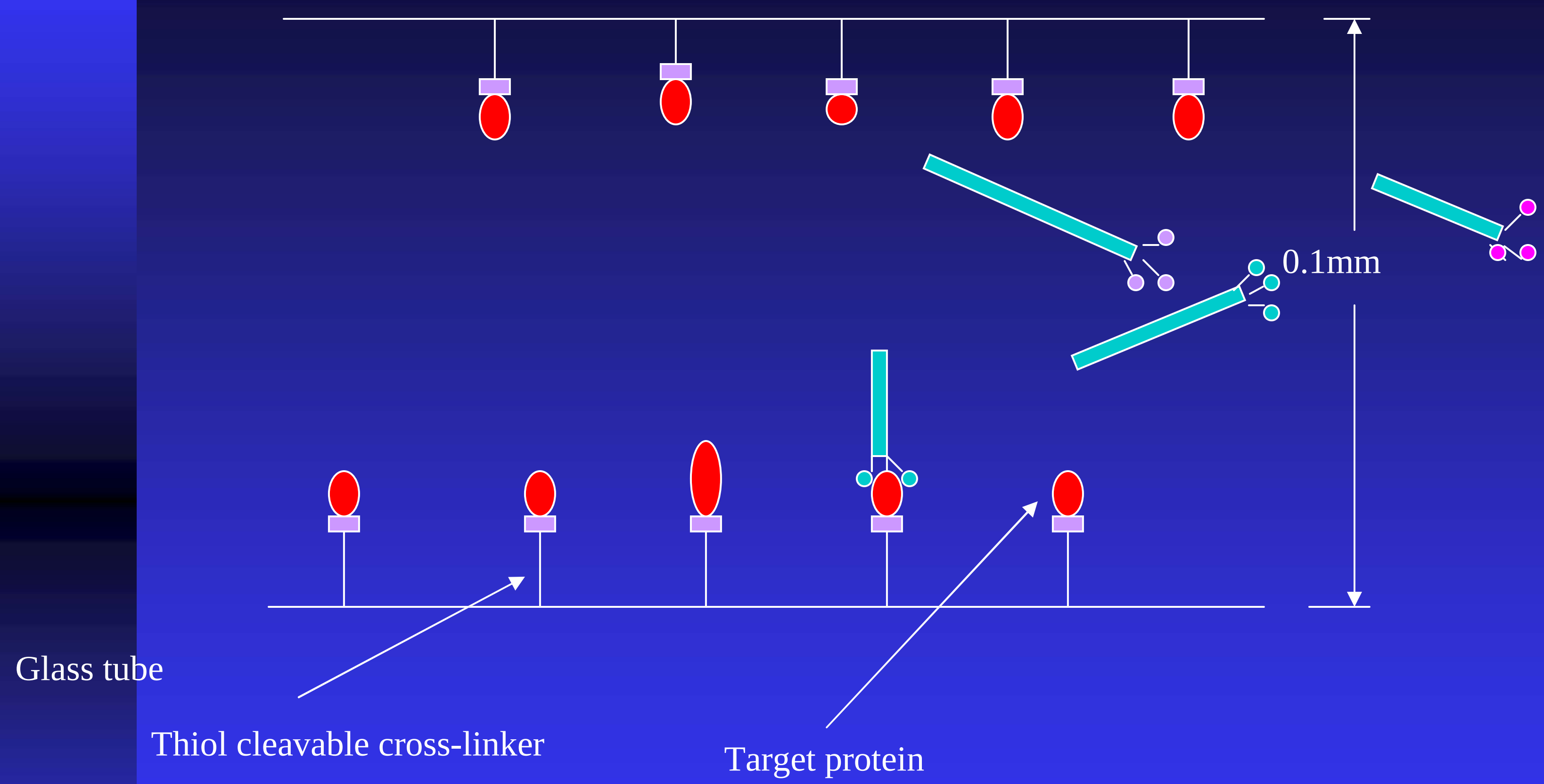

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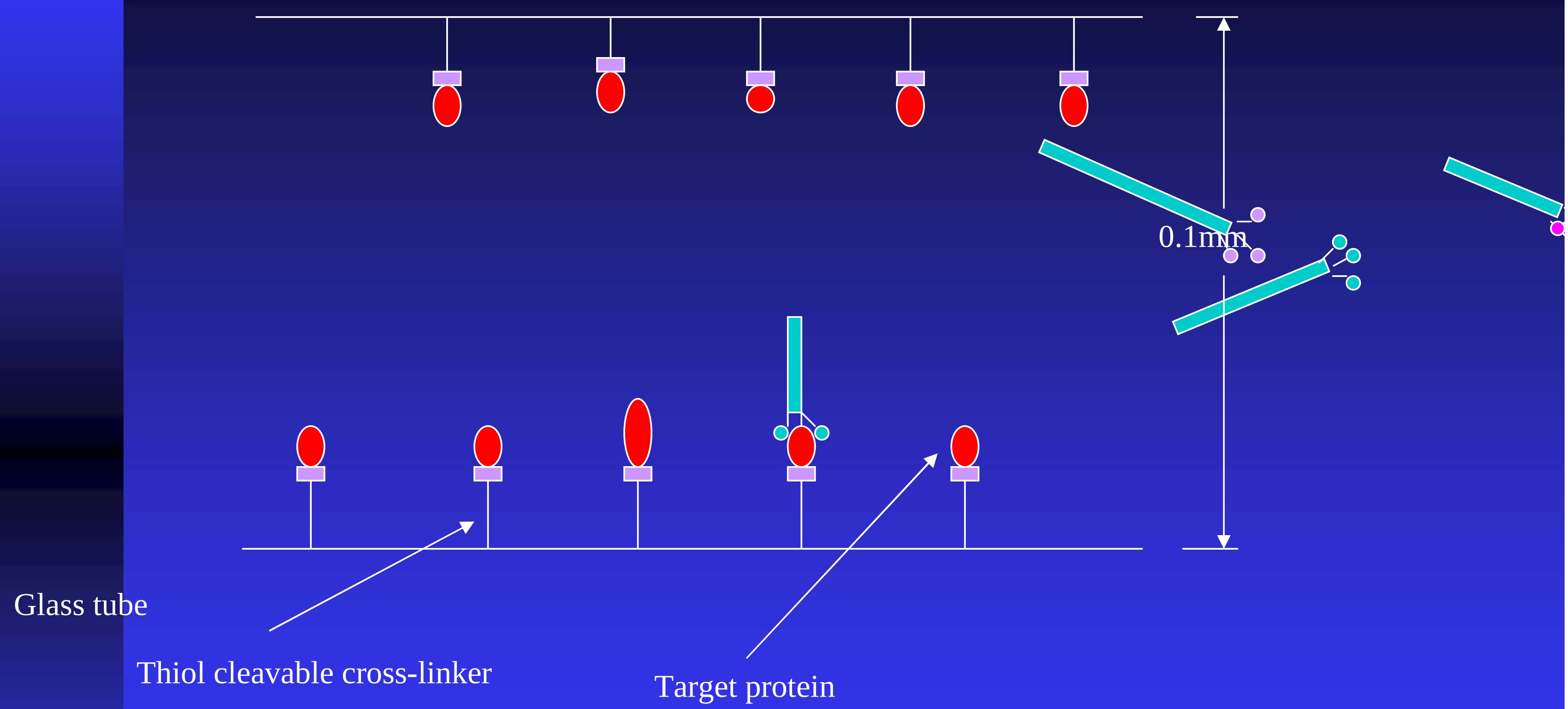

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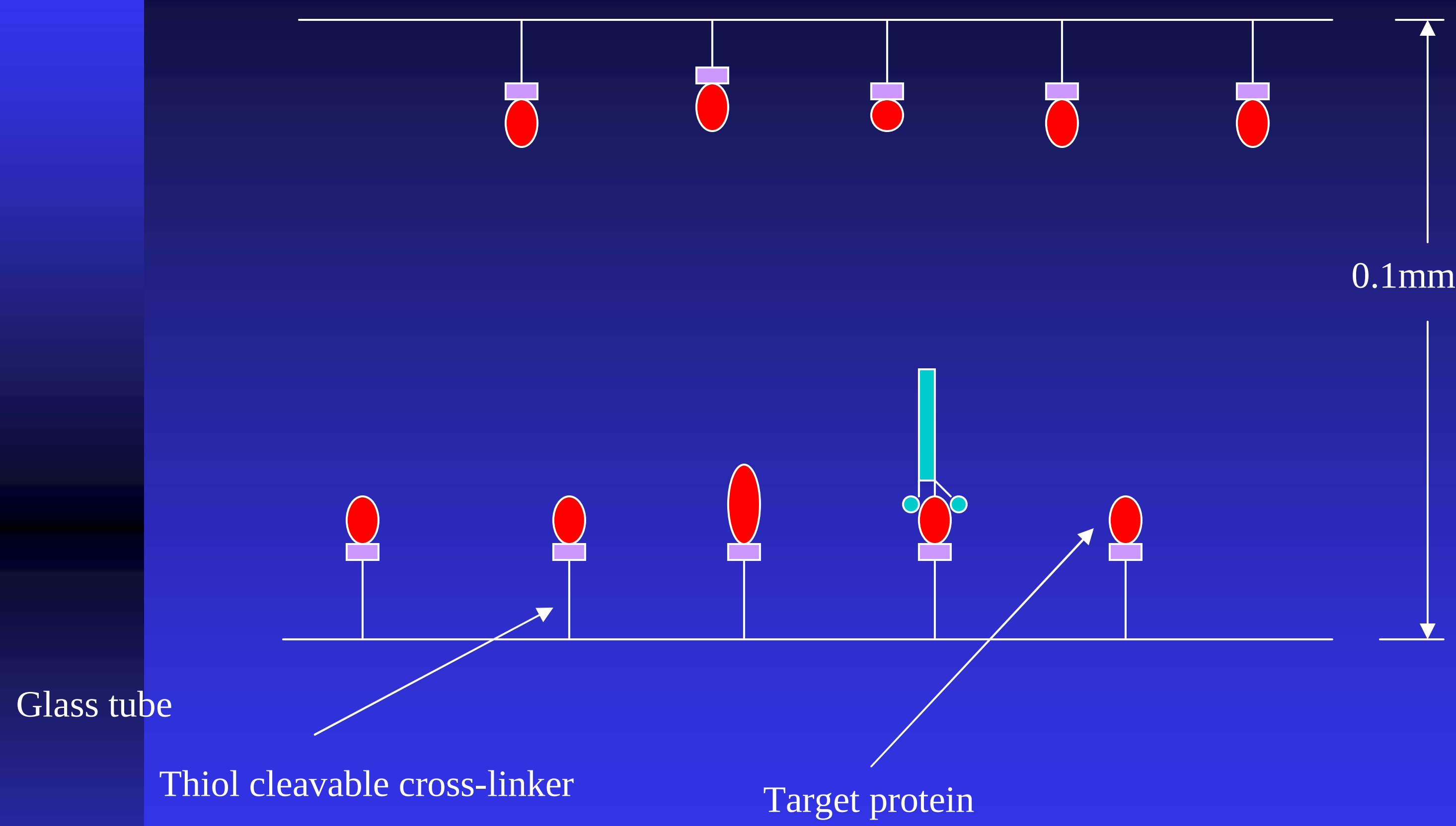

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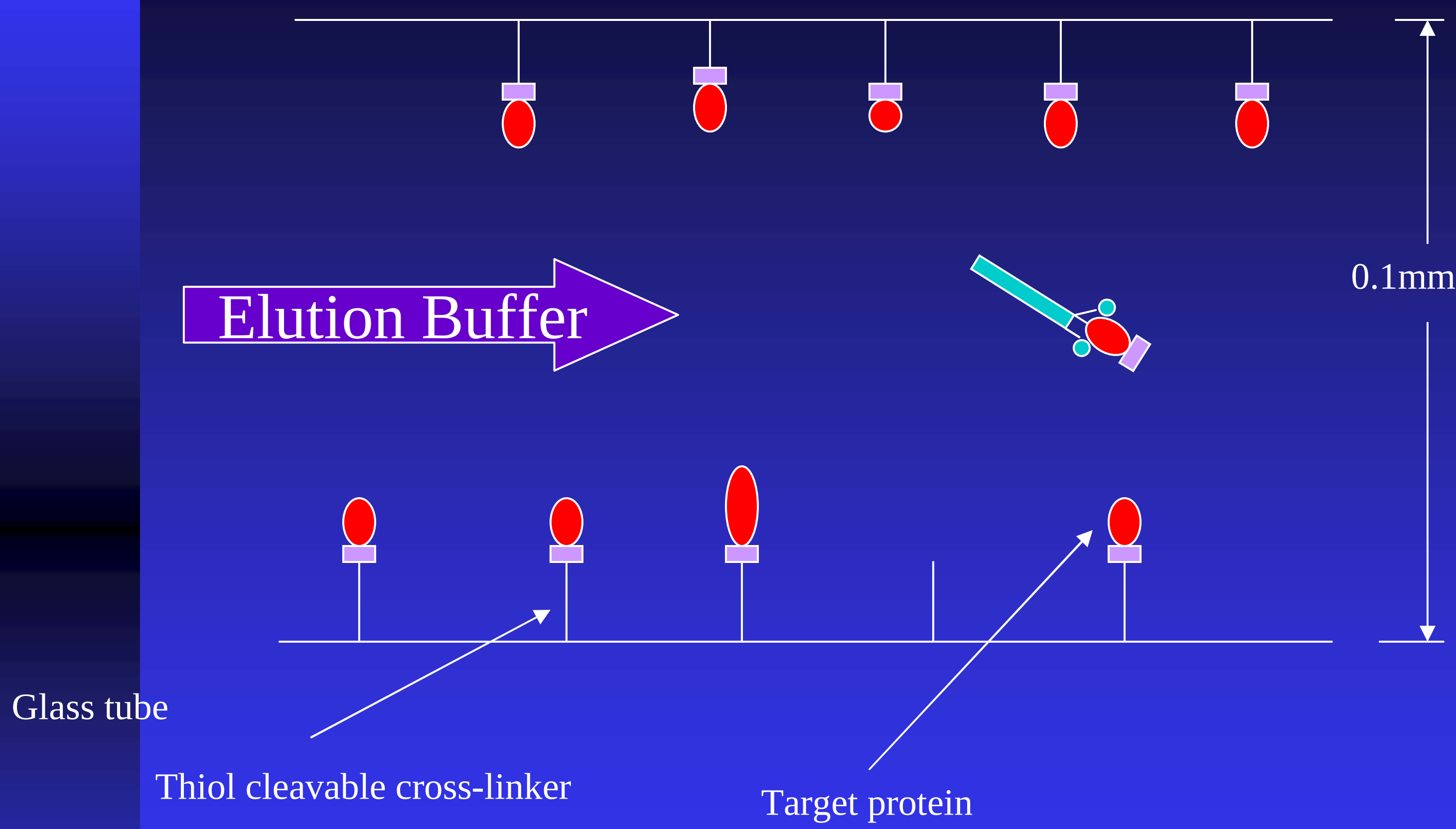

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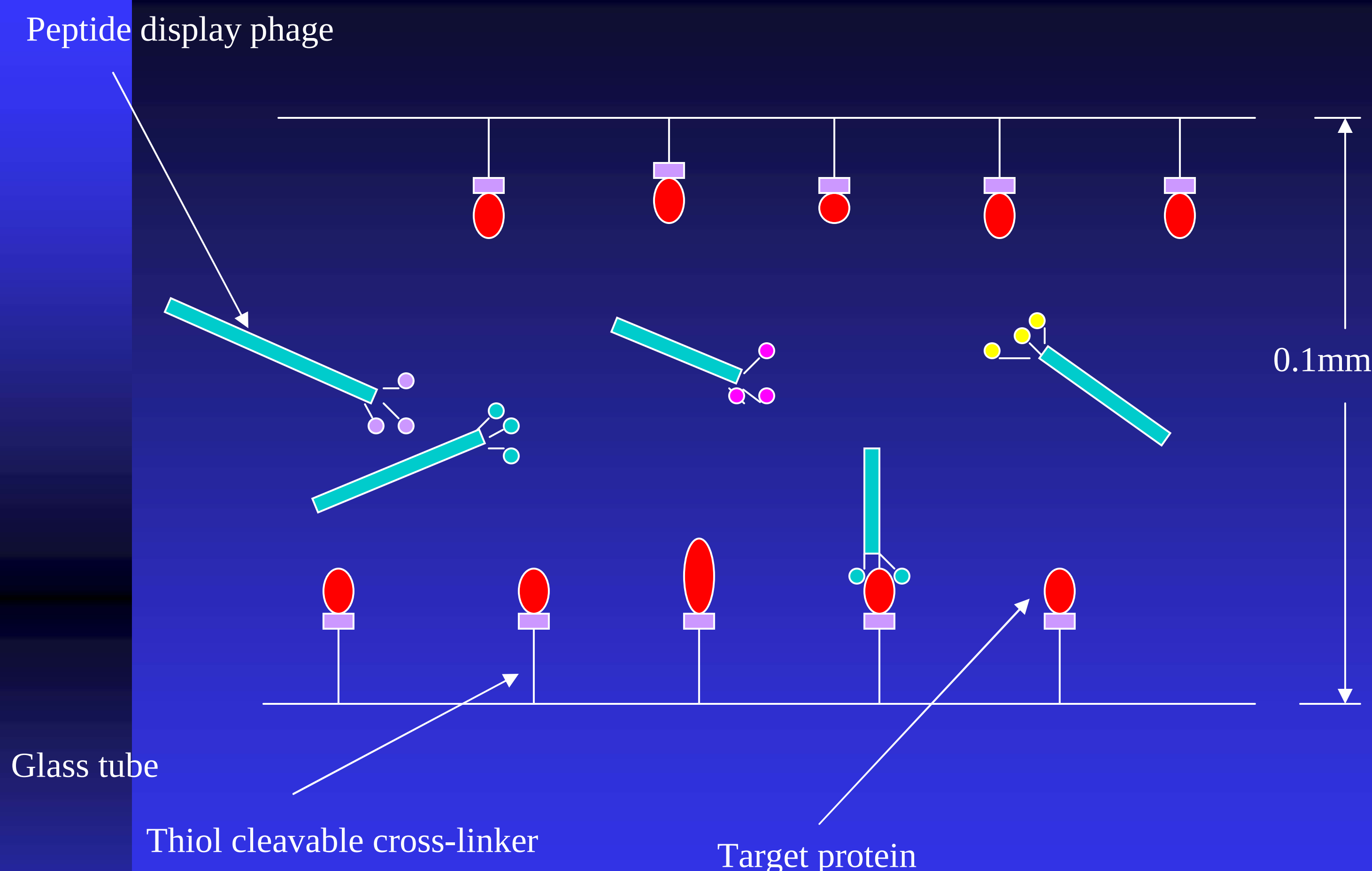


Advantages:

1. Can adjust flow rate = rigor of washing

2. Can remove all targets = cleavable cross-linkage agent

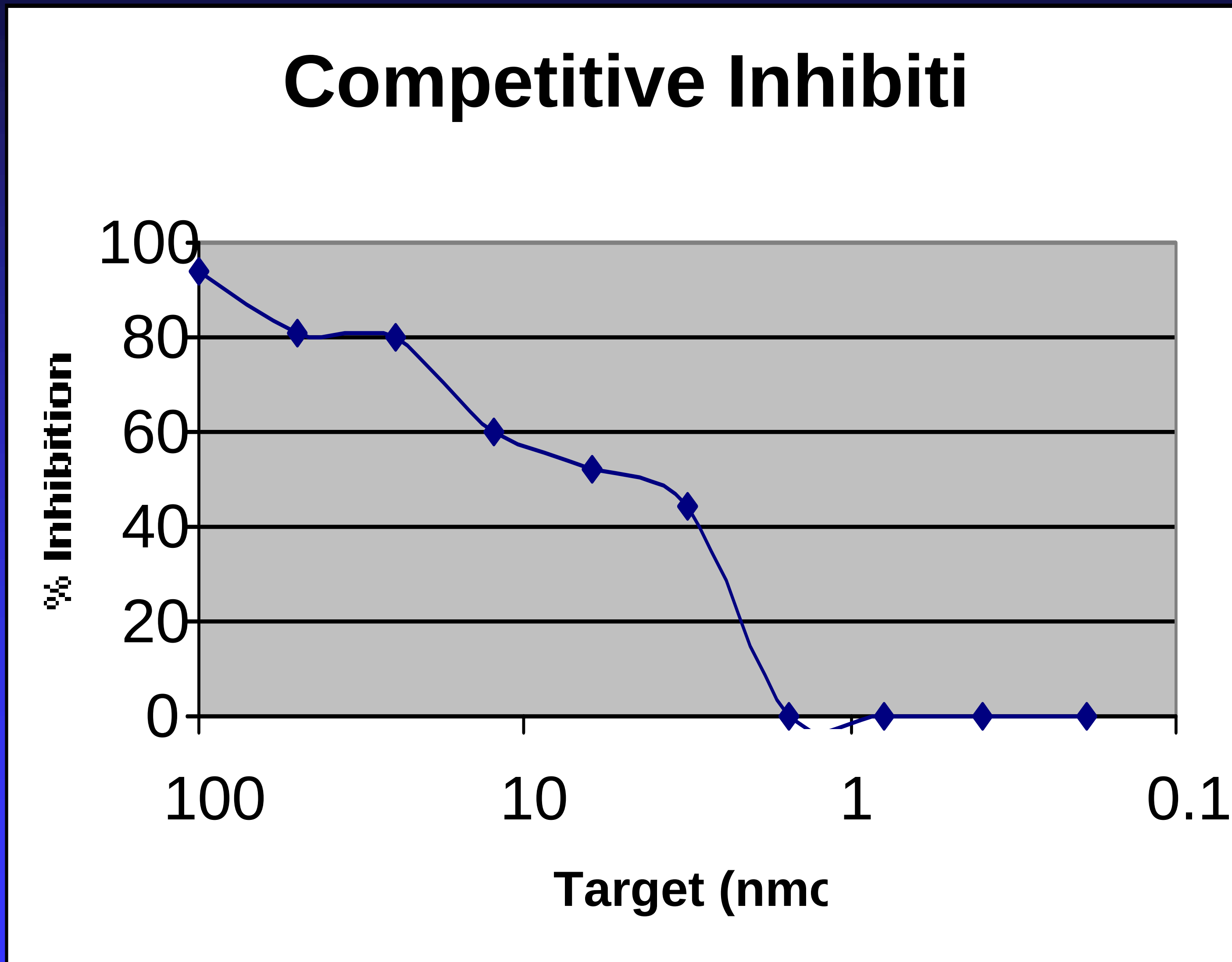
3. Can be automated for high-throughput

4. Can be adapted to small volumes (<5 ul)

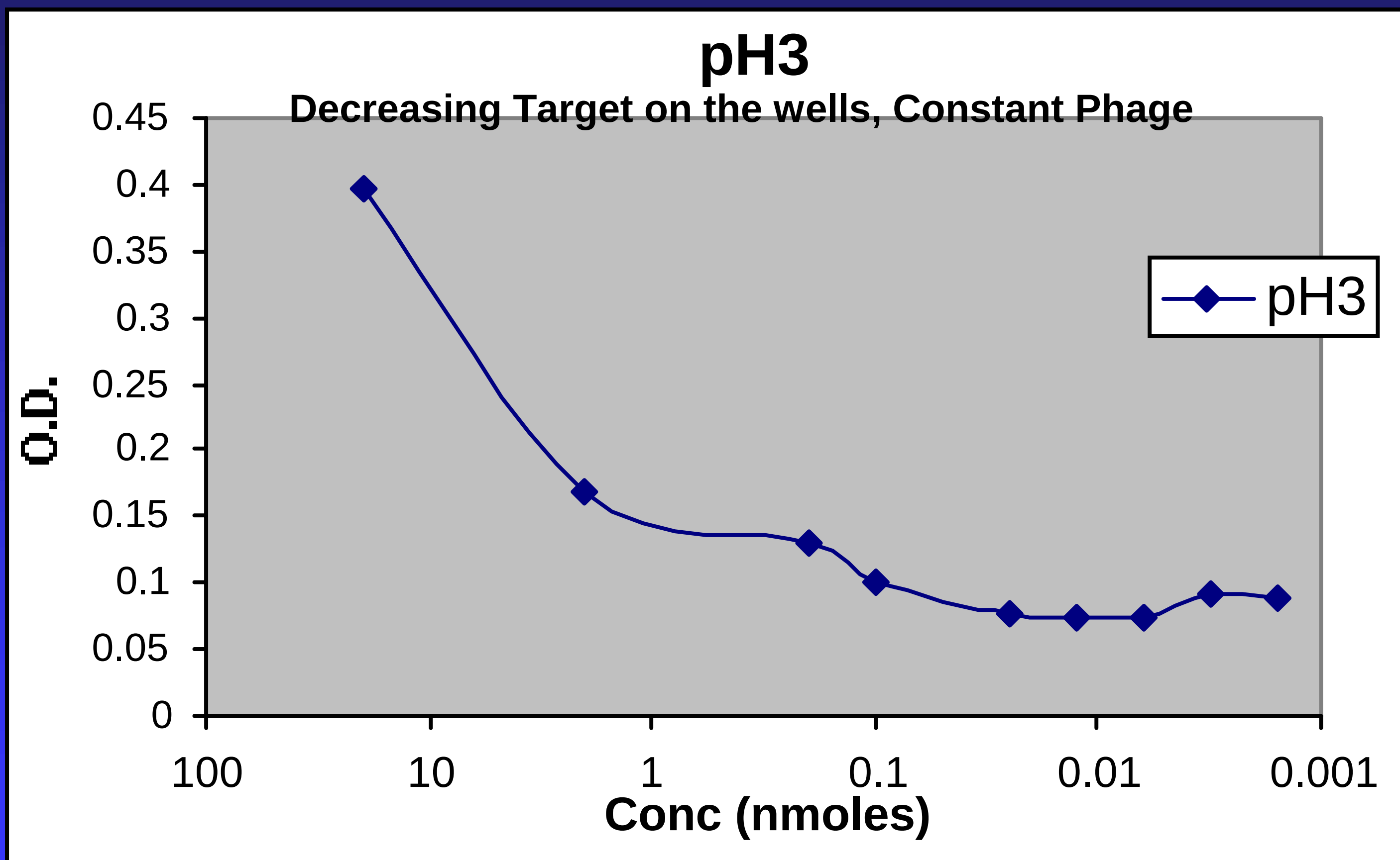
Sequences for peptides which bind to the Y. pestis F1 antigen

# 1	S F S L K P H A S L I R
# 2	G P N K F S L M H L F S
# 3	S F S L S S Y S A L L W
# 4	K F S L S P H T A W F L
# 5	K L S L N P H F M F Q S
# 6	F S L K N P T I A N T M
# 7	L I S V E P A S L S A H
# 8	S S L T L A P F S W S L
# 9	G P W F S L R H L S P Q
# 10	S H S W F R V N T L H L
# 11	G W F S T P L K W R M Q
# 12	S N F T L P F L K T F R
# 13	S W F T L H N L P N R P
# 14	N F S I N P R M M W P V
# 21	F S I K H P W P F F L P
# 28	F S L K L P Y W Q R T F

Use of free F1 antigen to compete with phage in its binding to F1 attached to a plate

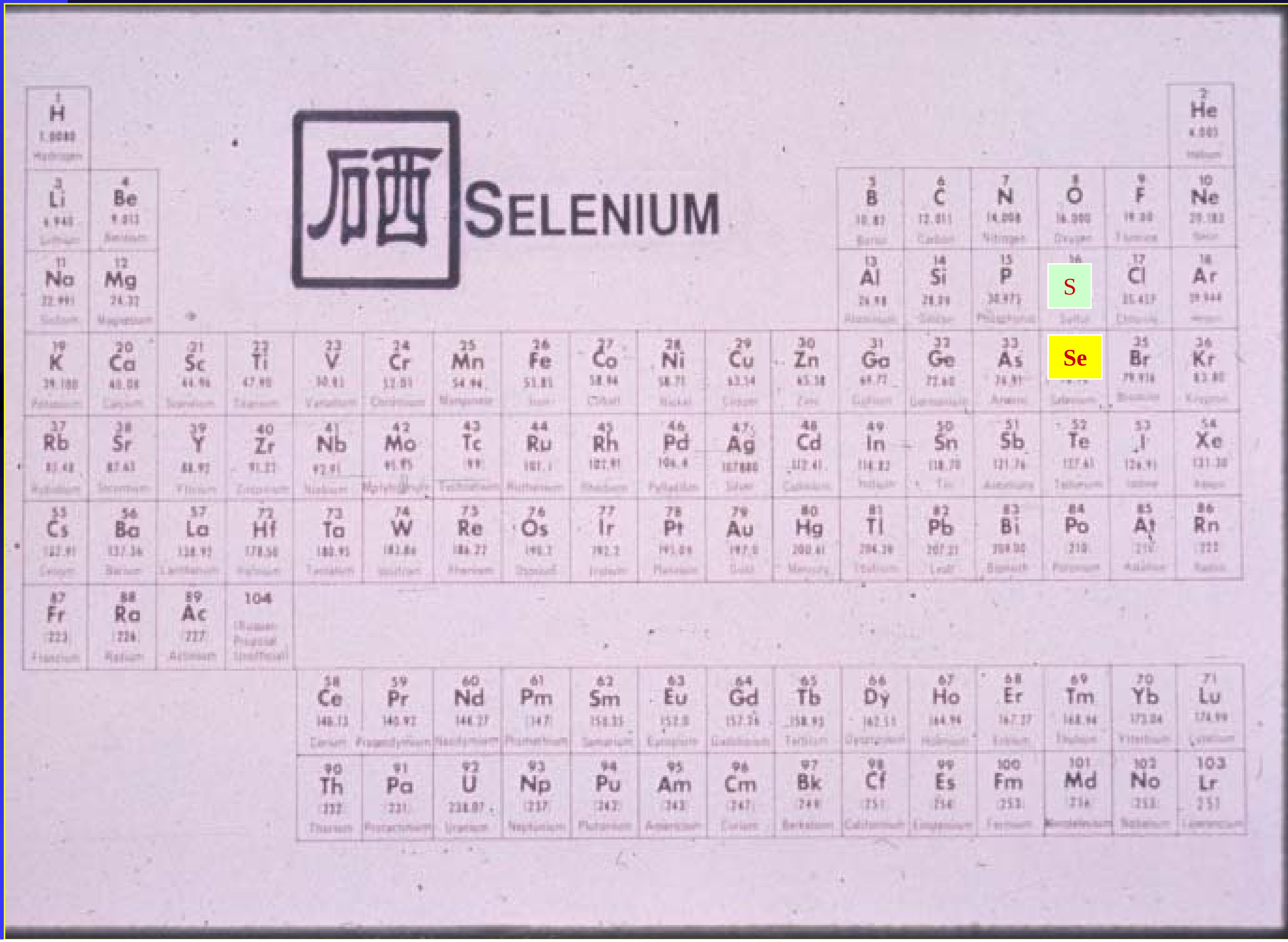


Effect of decreasing the amount of F1-antigen on the plate



ORGANO-SELENIUM

Selenium Chemistry is a lot like that of Sulfur



Selenium has a Good Side and a Dark Side

Selenium is essential for your diet

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- You need approximately 200 ug/day.

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- A selenium amino acid has its own genetic code.

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- From the sequencing of the human genome it is found that selenium is incorporated into 25 different proteins.

Selenium is essential for your diet

- You need approximately 200 ug/day.
- A selenium amino acid has its own genetic code.
- From the sequencing of the human genome it is found that selenium is incorporated into 25 different proteins.
- Many of these selenium containing proteins function to destroy oxygen radicals.

The Dark Side: If you eat too much Selenium it will kill you

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- 3 mg/day will make you sick

The Dark Side: If you eat too much Selenium it will kill you

- 3 mg/day will make you sick
- 30 mg/day will kill you

We Work on the Dark Side

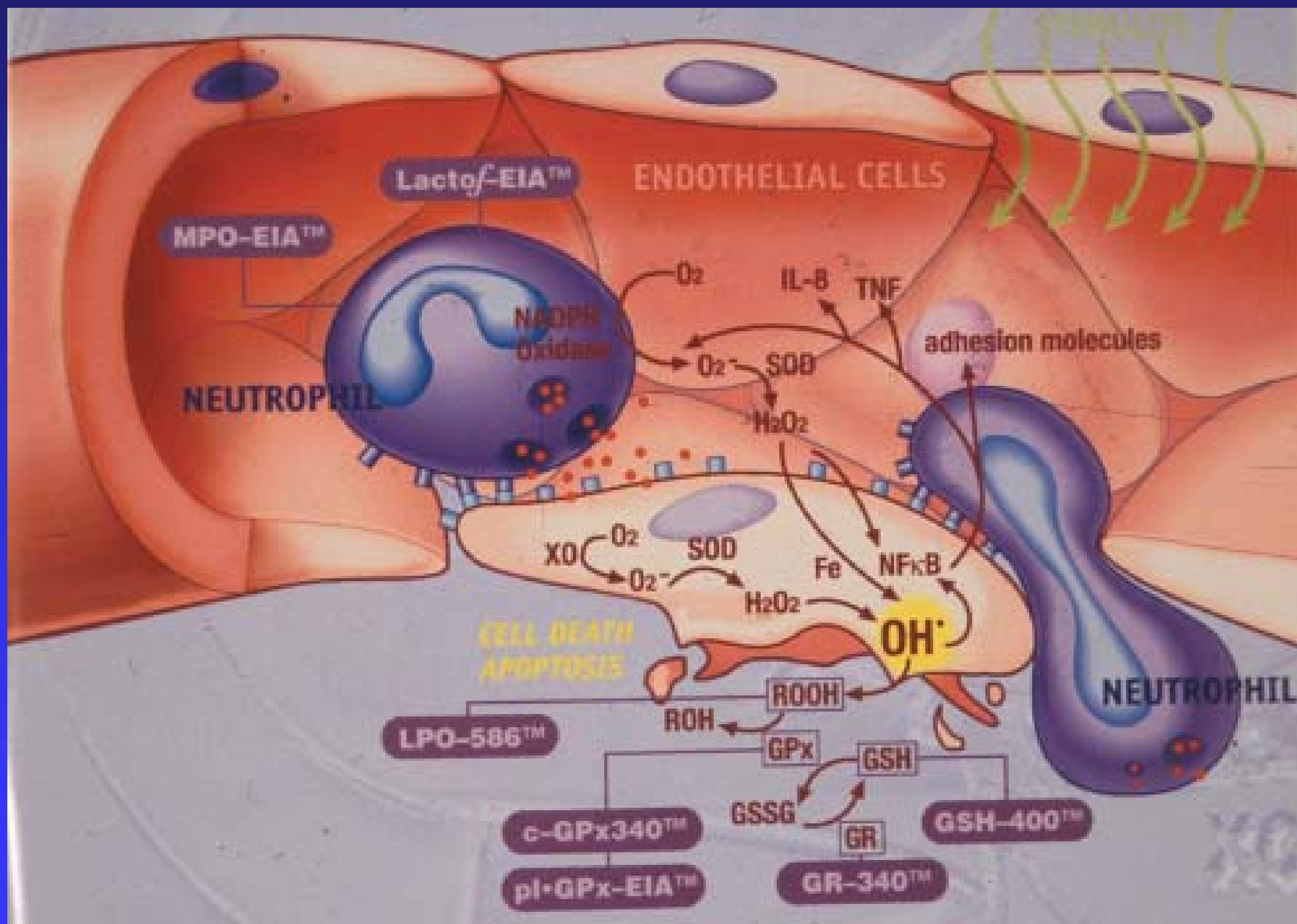
Selenium can catalyze the formation of superoxide radicals

Selenium can catalyze the formation of superoxide radicals

$$R\text{-}Se^{-} + O_2 \rightarrow RSe^{\cdot} + O_2^{\cdot -}$$

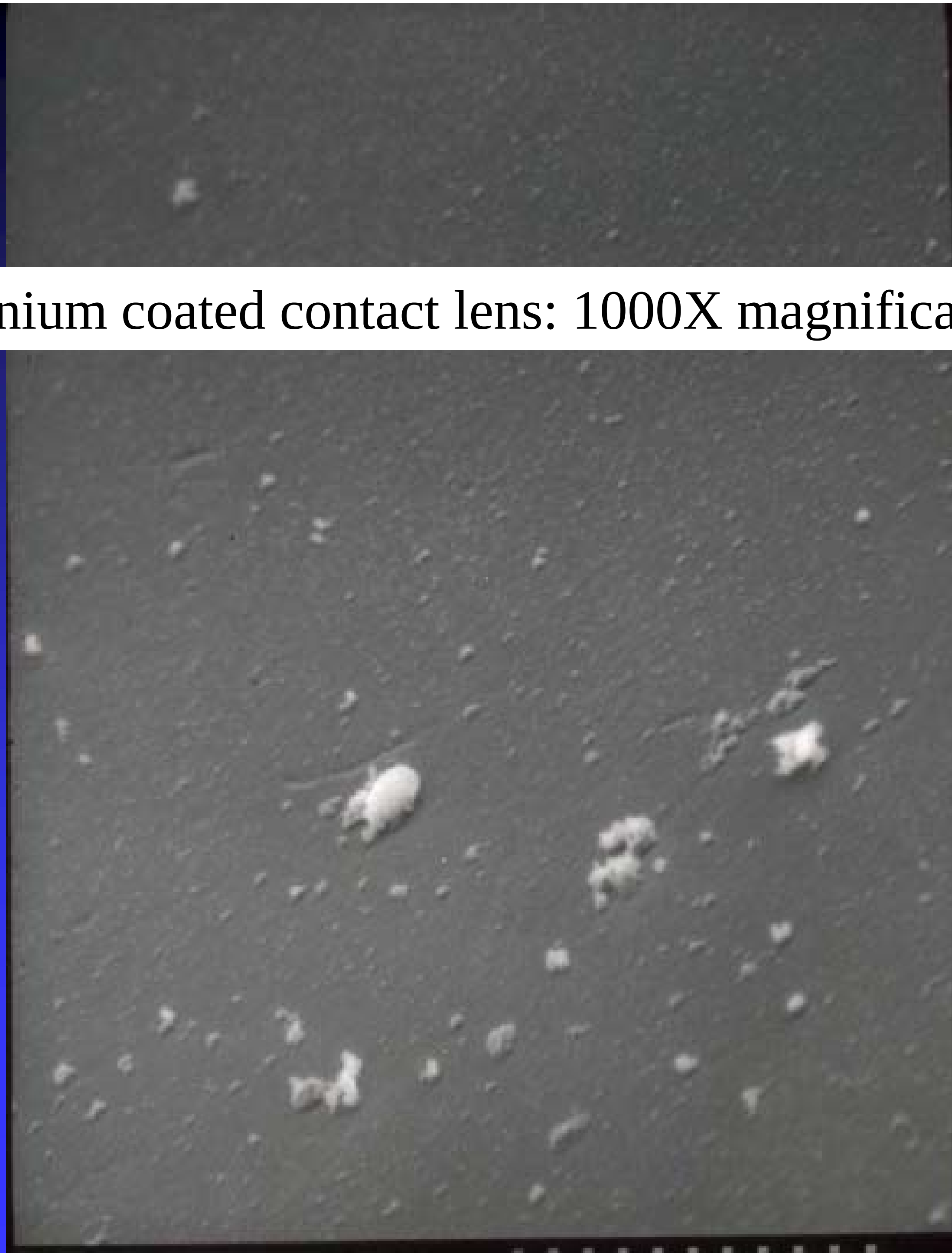
$$R\text{-}Se^{\cdot} + 2R'\text{-}S^{-} \rightarrow R\text{-}Se^{-} + R'\text{-}S\text{-}S\text{-}R'$$

Superoxide killing mechanism

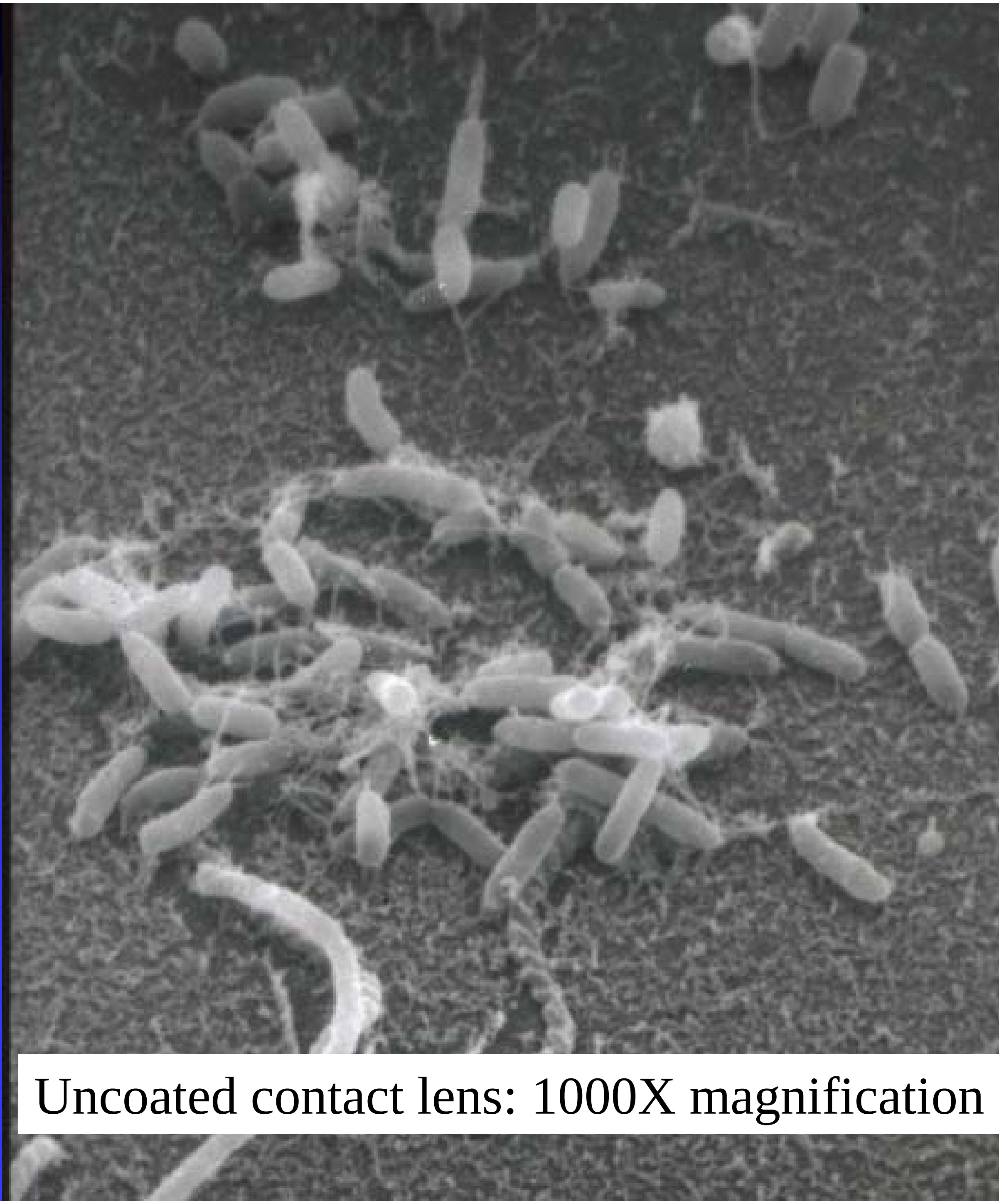


Selenium covalently attached to a contact lens.

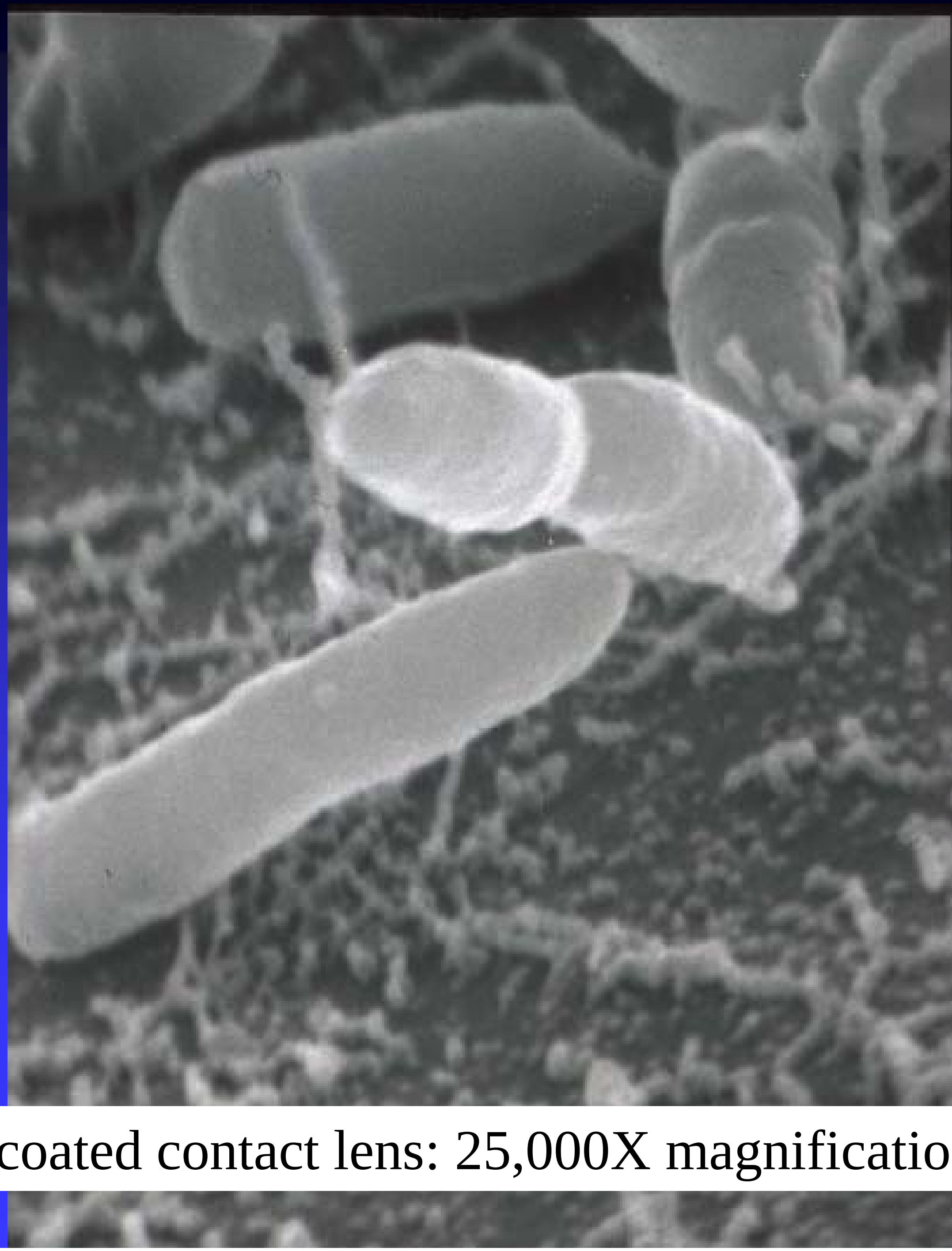
Contact lens placed in broth with Pseudomonas aeruginosa for 4 days



Selenium coated contact lens: 1000X magnification



Uncoated contact lens: 1000X magnification



Uncoated contact lens: 25,000X magnification

Hypothesis

Selenium labeled peptides and selenium labeled bacterial viruses (phage) can be produced that can selectively bind to the surface of a pathogenic bacteria and inactive them through the generation of superoxide radicals on their surface.

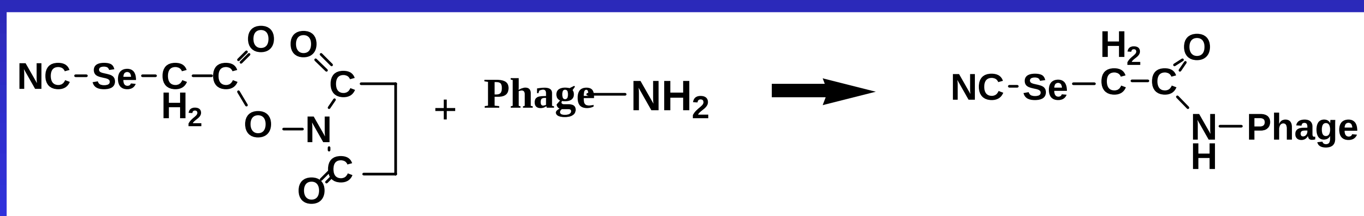
Initial experiments

- Initial experiments were done with labeled bacterial viruses

Filamentous Bacteriophage

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

Attachment of Selenium to Phage



Bacteria with F1 protein expressed on their surface

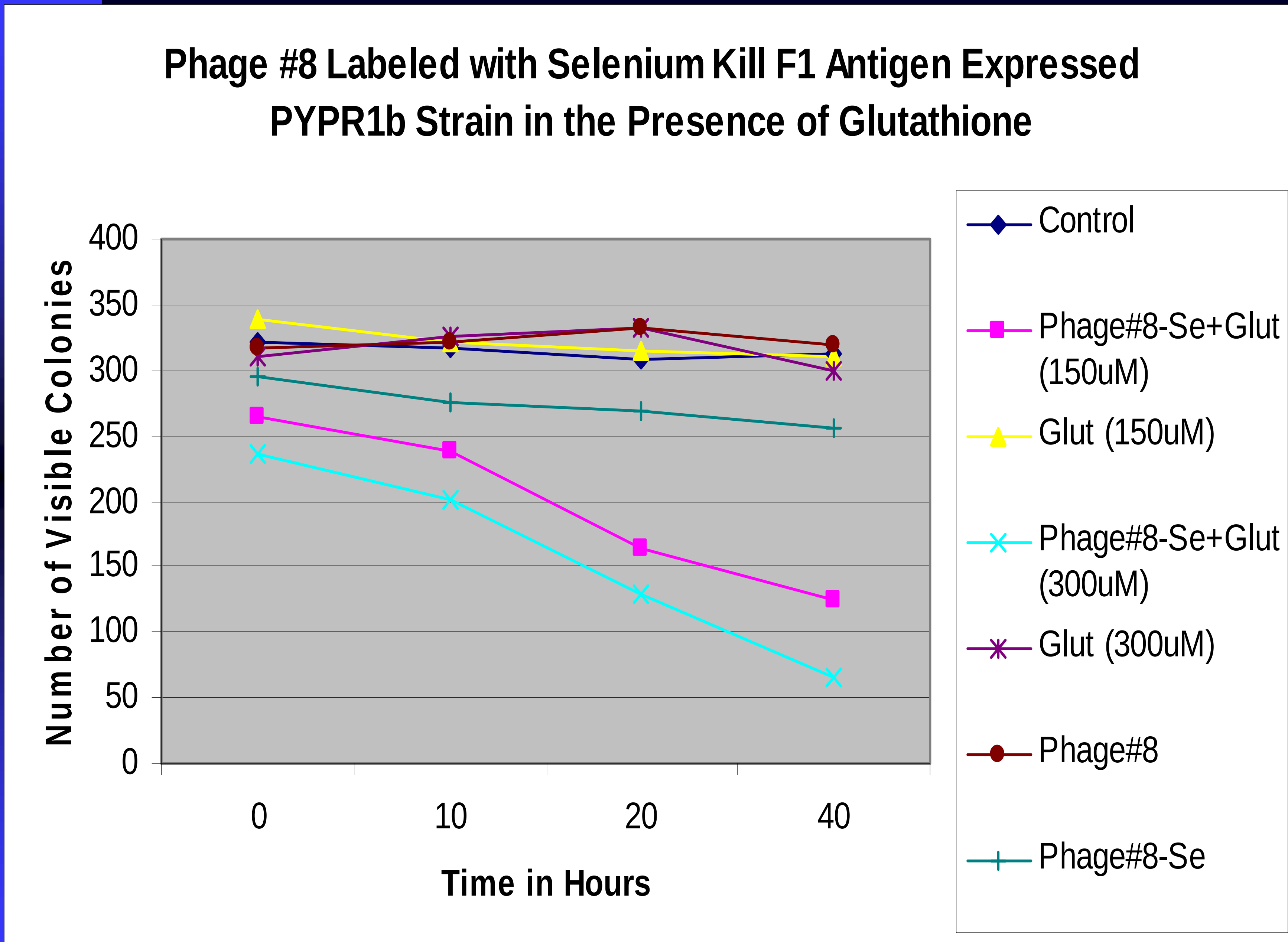
Use of phage which show F1 specific binding

Bacteria with F1 protein expressed on their surface

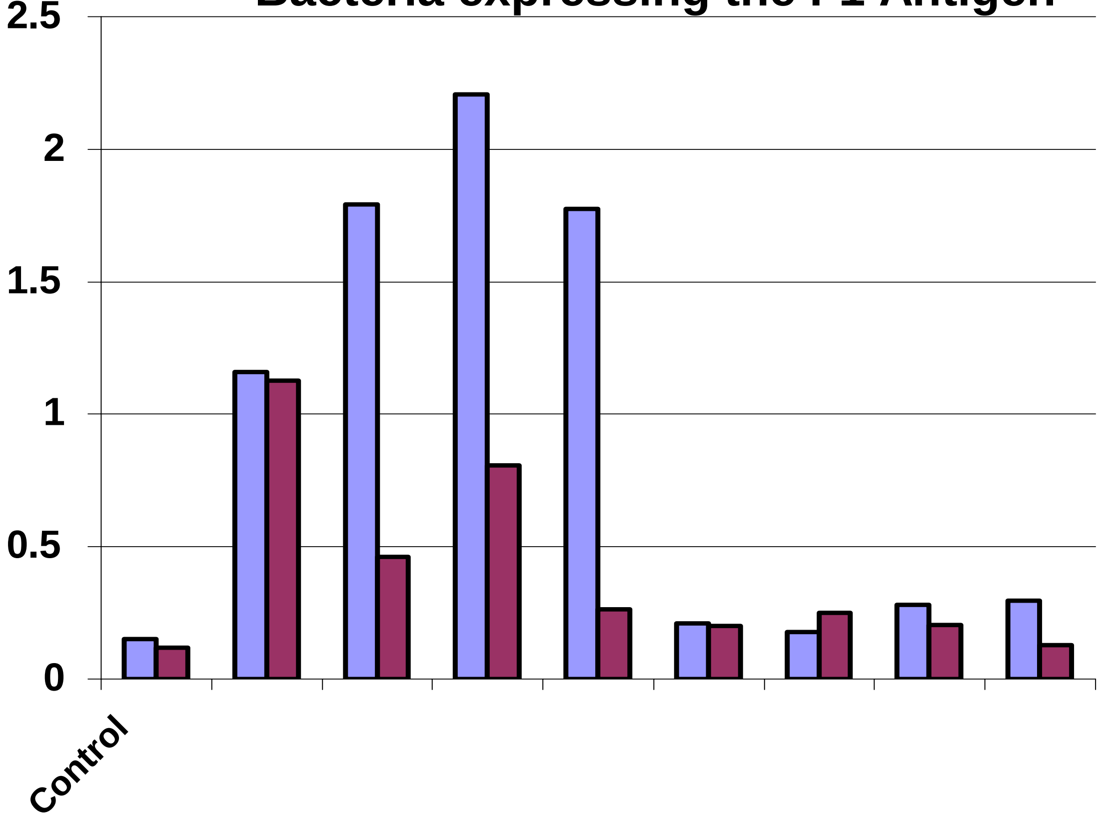
- Use of phage which show F1 specific binding
- Attach selenium to the specific phage

Bacteria with F1 protein expressed on their surface

- Use of phage which show F1 specific binding
- Attach selenium to the specific phage
- Test selenium labeled phage with and with out an external source of sulfur (glutathione) by mixing with bacteria and then plating to determine number of live bacteria


Phage #8 Labeled with Selenium Kill F1 Antigen Expressed PYPR1b Strain in the Presence of Glutathione
Number of Visible Colonies
400
350
300
250
200
150
100
50
0
0
10
20
40
Time in Hours
Control
Phage#8-Se+Glut (150uM)
Glut (150uM)
Phage#8-Se+Glut (300uM)
Glut (300uM)
Phage#8
Phage#8-Se

Specific F1 YP Phage #8 (10^{11}) Inhibit F1 Mouse Monoclonal Antibody in Competition Binding Assay with Bacteria expressing the F1 Antigen



Peptide #8 From the Phage Binding Studies

Ser-Ser-Leu-Thr-Leu-Ala-Pro-Phe-Ser-Trp-Ser-Leu

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Ser-Ser-Leu-Thr-Leu-Ala-Pro-Phe-Ser-Trp-Ser-Leu

Selenium was covalently attached to this peptide

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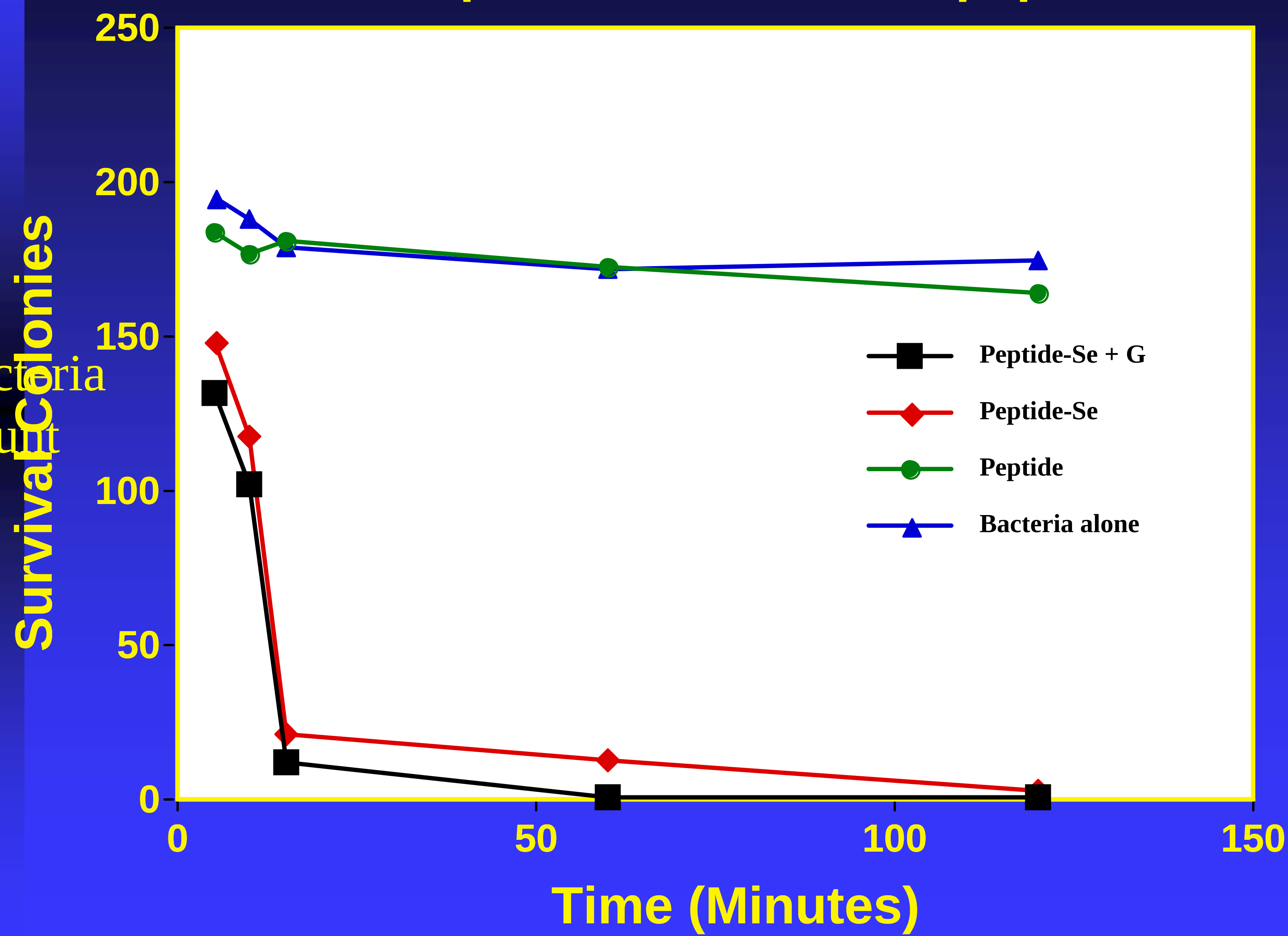
Selenium was covalently attached to this peptide

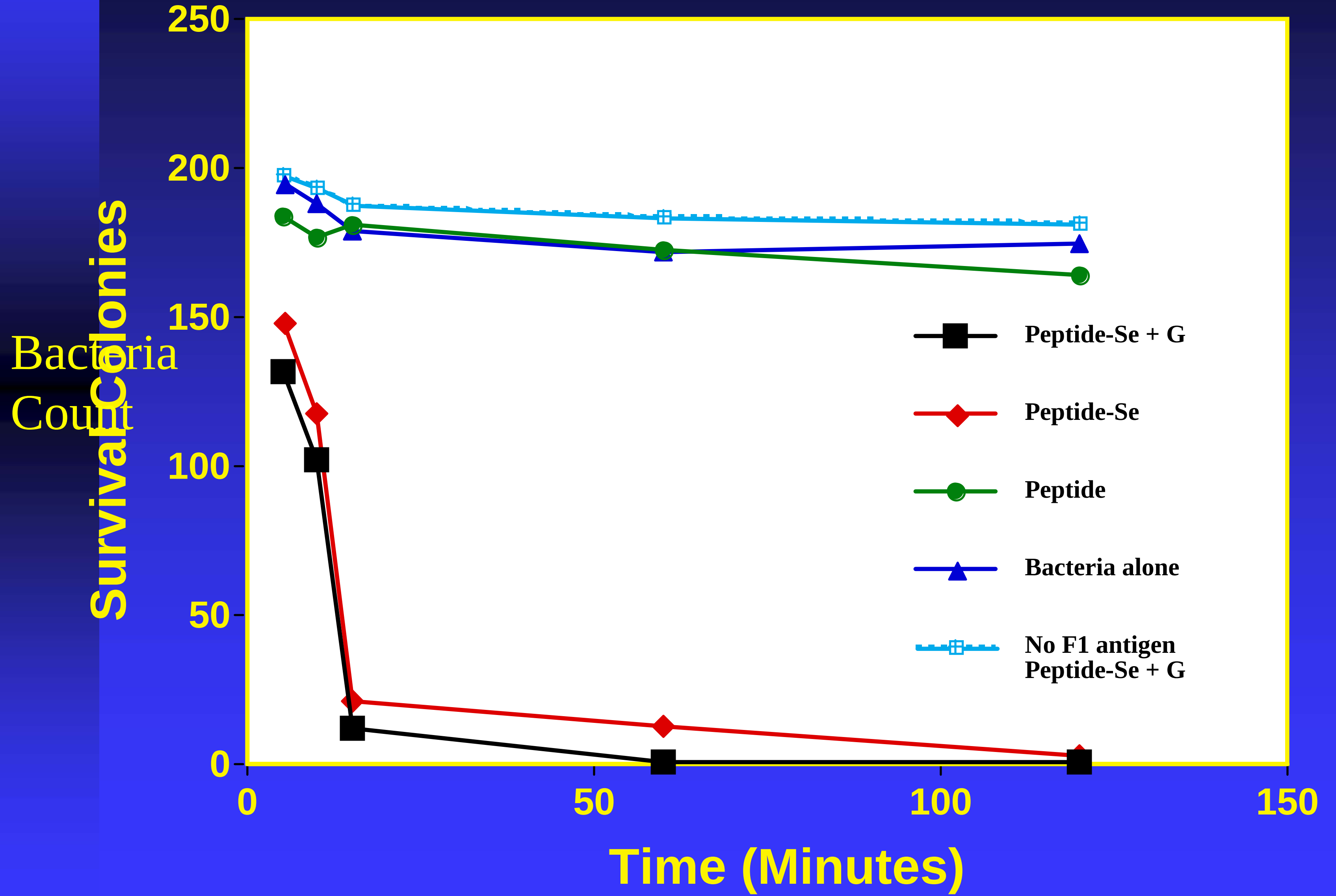
The seleno-peptide was tested at 1 uM

Survival of Bacteria expressing F1 antigen In the presence of Seleno-peptide

Bacteria Count



Survival of Bacteria expressing F1 antigen In the presence of Seleno-peptide



Conclusions

- Selenium can be attached to a bacterial virus which can target and bind to a specific bacteria.

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- A selenium labeled virus targeted for a specific bacteria can kill the bacteria.

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- Selenium can be attached to a bacterial virus which can target and bind to a specific bacteria.
- A selenium labeled virus targeted for a specific bacteria can kill the bacteria.
- The selenium labeled virus killing of the bacteria is promoted by glutathione.
- Bacterial killing with a selenium labeled phage takes about 40-60 hours using 10^{11} phage.

Conclusions - Peptide

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- A seleno-peptide can kill 95% of a specific bacteria in 15 minutes and all of the bacteria in less than 1 hour.

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Conclusions - Peptide

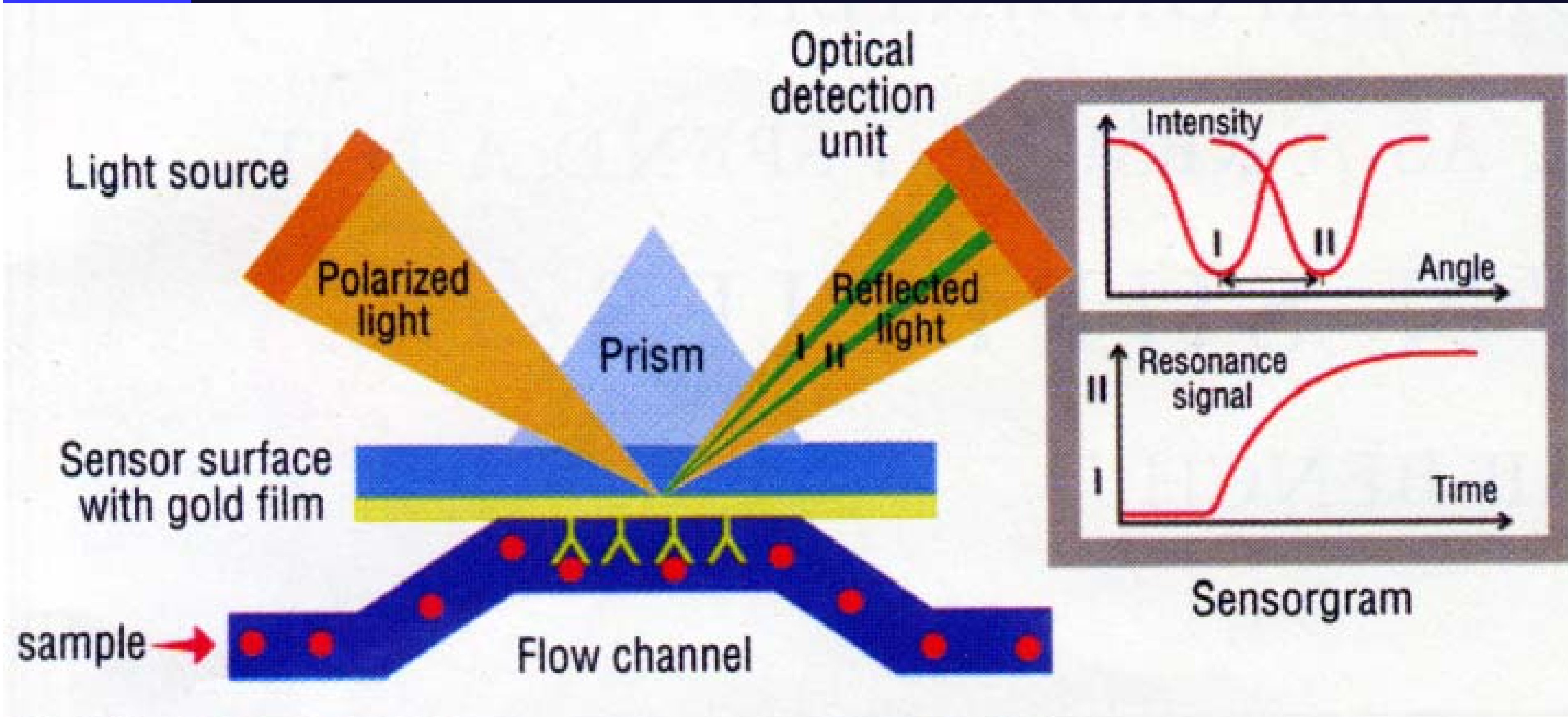
- Selenium can be attached to a peptide which can target and bind to a specific bacteria.
- A seleno-peptide can kill 95% of a specific bacteria in 15 minutes and all of the bacteria in less than 1 hour.
- The seleno-peptide can kill at 1 micromolar concentration.
- The seleno-peptide will not kill bacteria that do not express the required binding protein.

Future Studies

- Bacterial killing studies in vivo

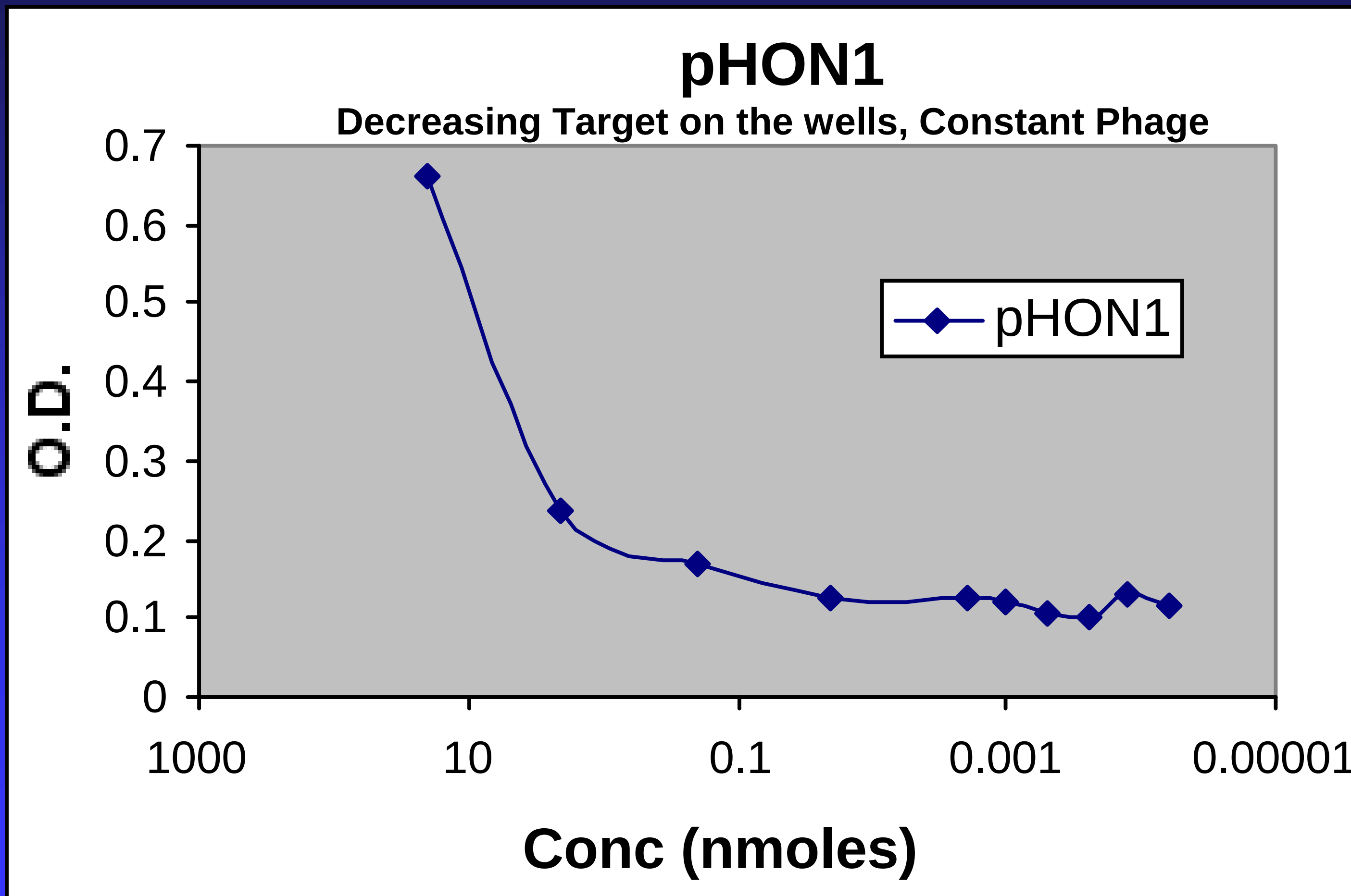
Future Studies

- Bacterial killing studies in vivo.
- Testing and design of new seleno-peptides which bind to other targets (new bacteria).

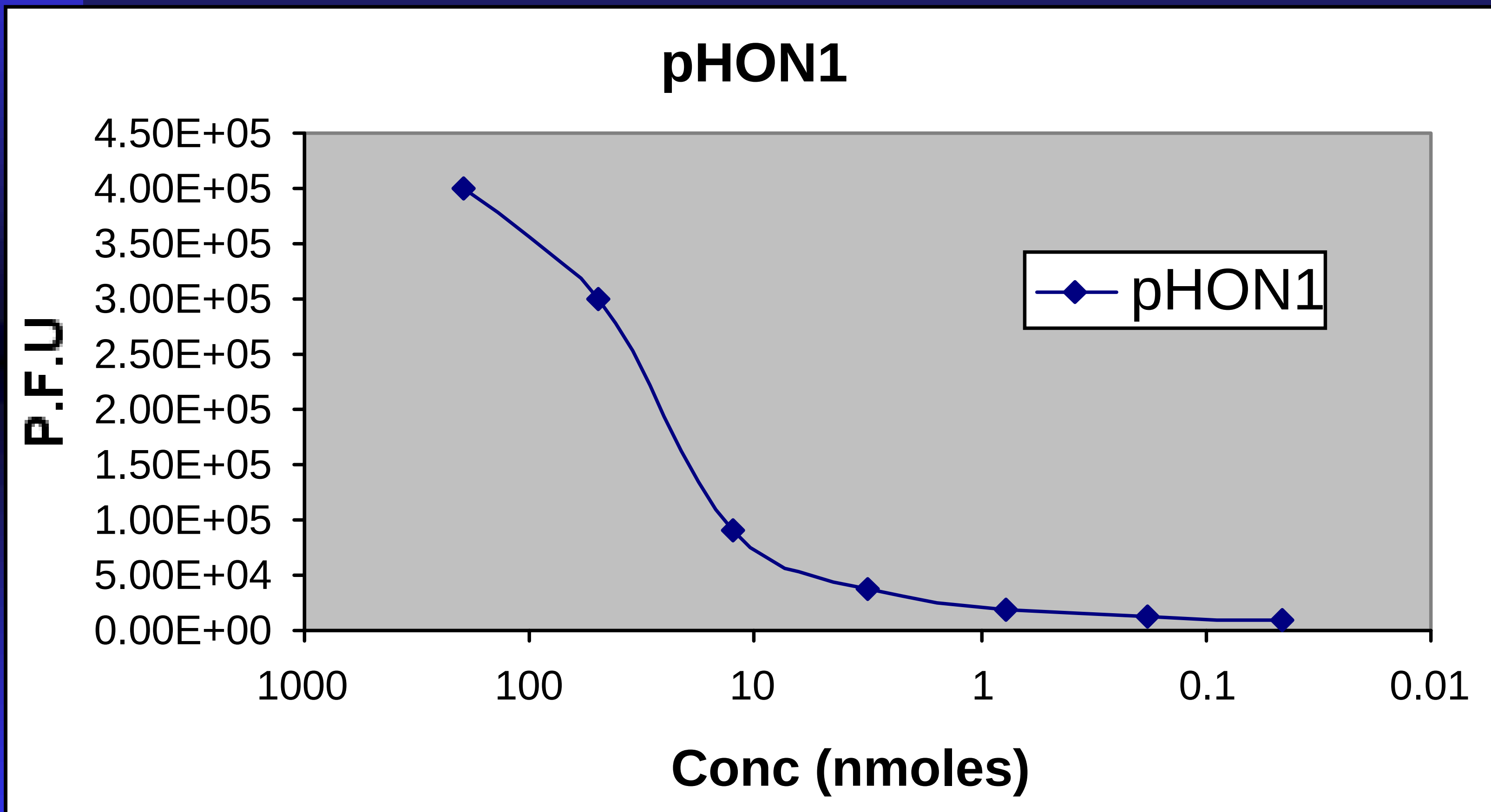

Optical
detection
unit
Light source
Polarized
light
Prism
Reflected
light
I
II
Intensity
I
II
Angle
Resonance
signal
II
I
Time
Sensorgram
Sensor surface
with gold film
sample
Flow channel

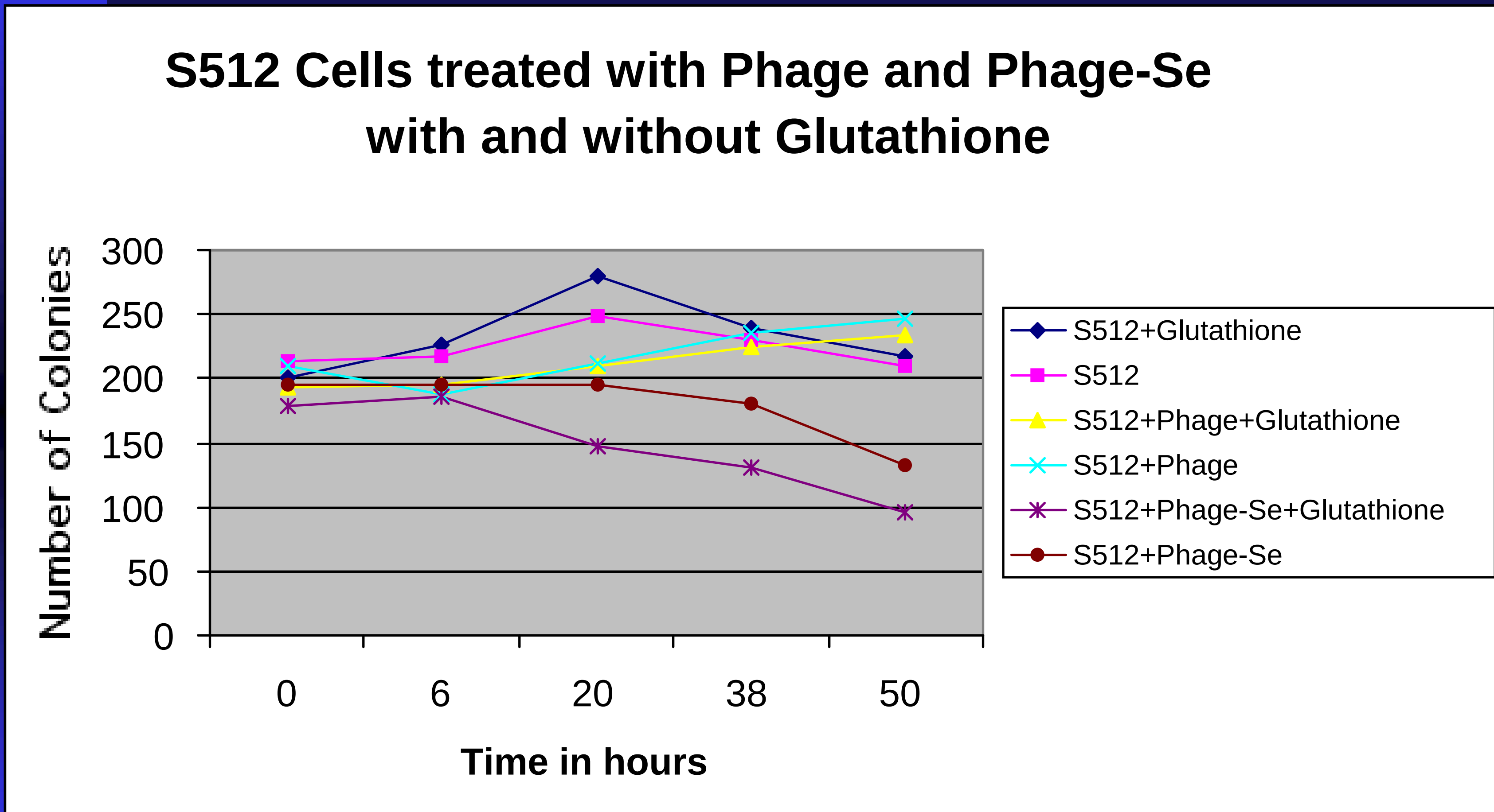
QuickTime™ and a
GIF decompressor
are needed to see this picture.

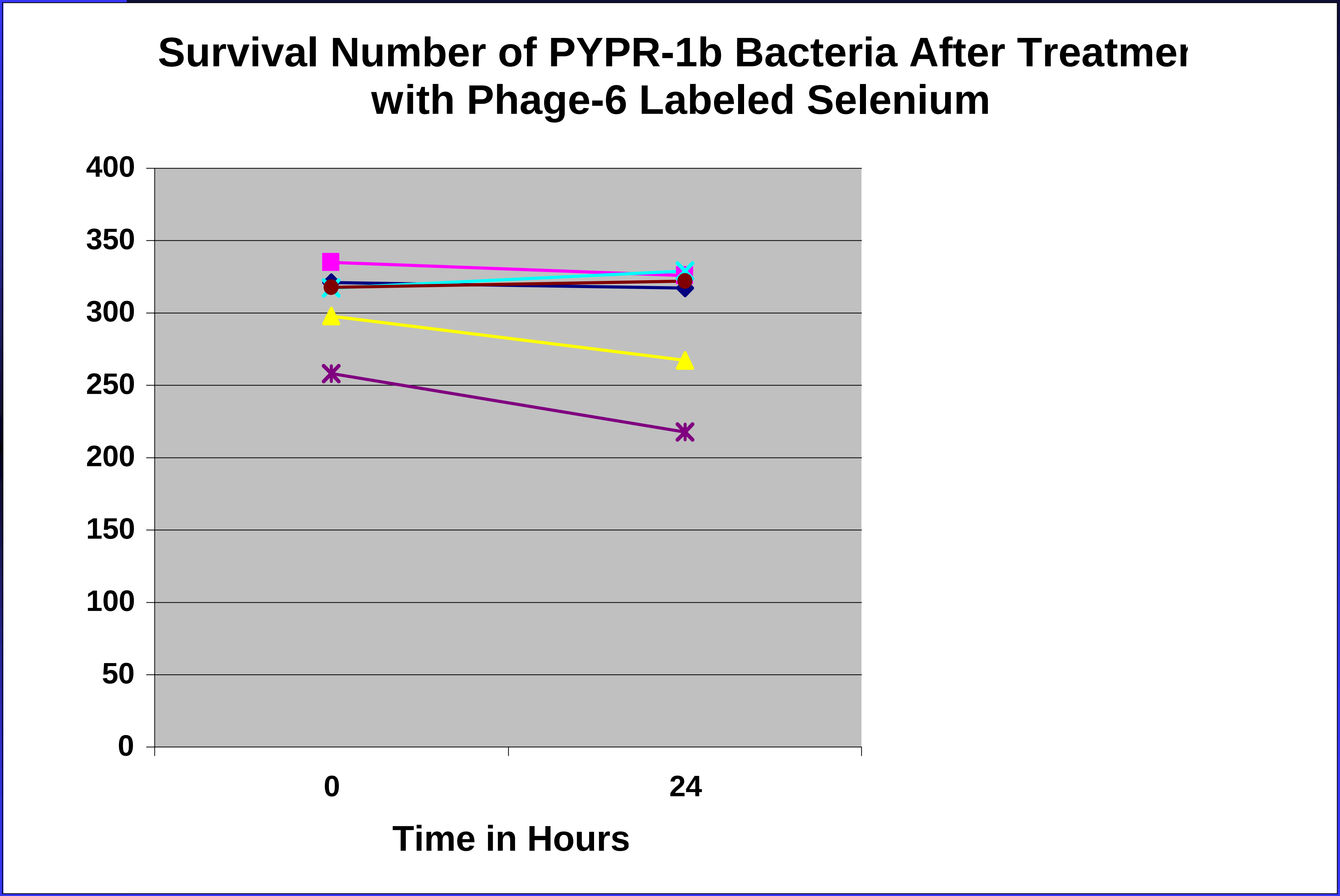
Effect of decreasing the amount of F1-antigen on the plate



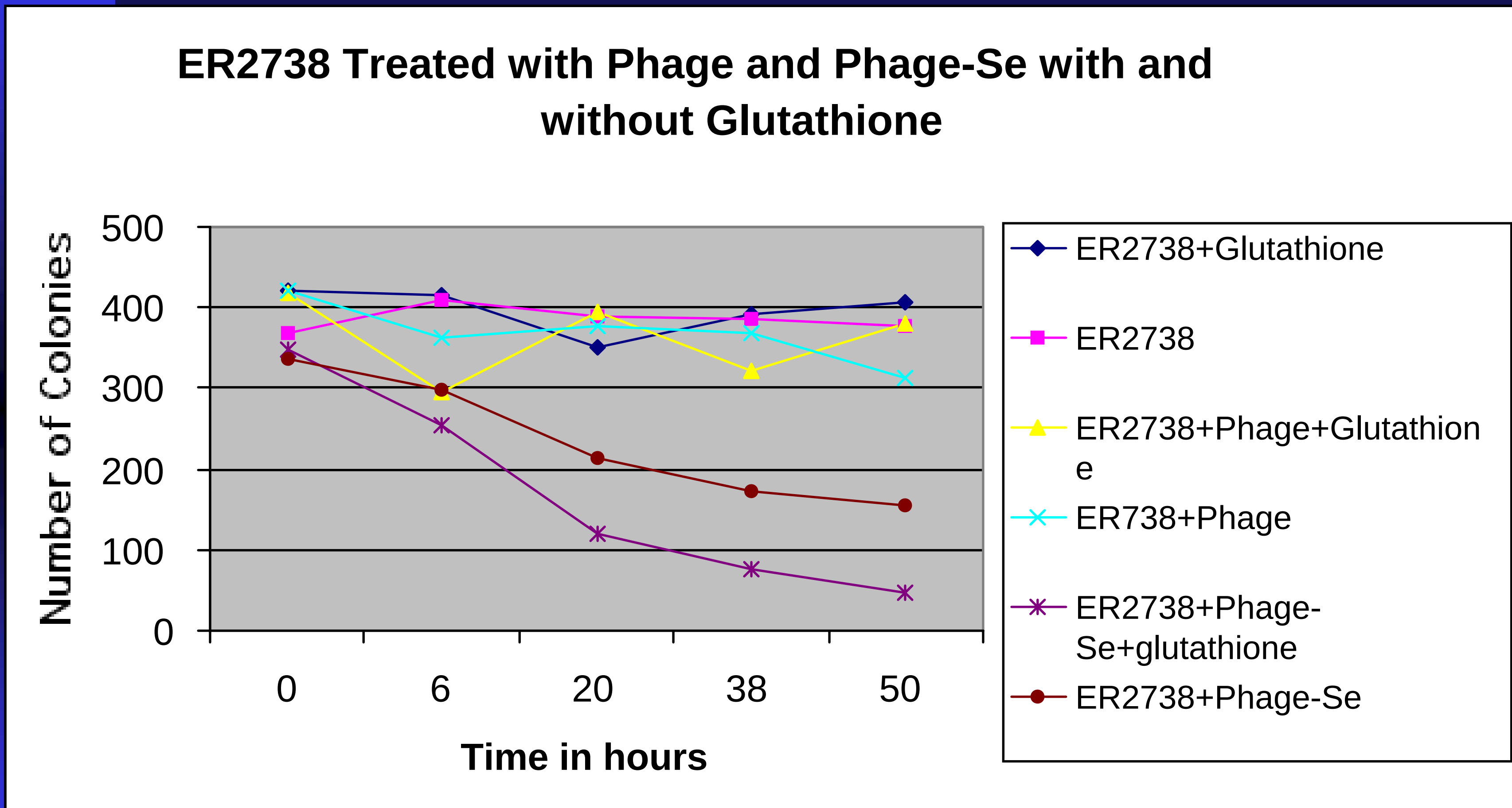
Competition between phage and free F1 antigen for antigen attached to the plate

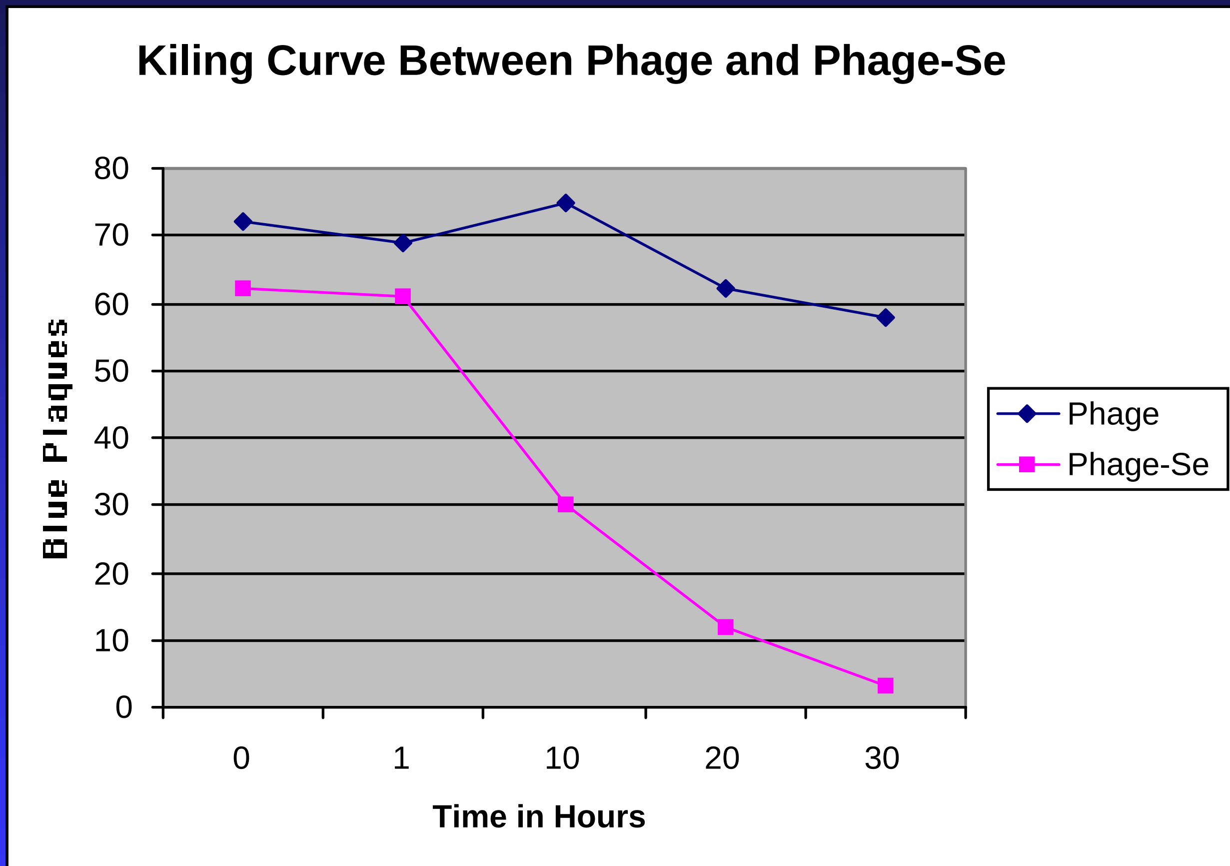



S512 Cells treated with Phage and Phage-Se
with and without Glutathione
Number of Colonies
300
250
200
150
100
50
0
0
6
20
38
50
Time in hours
S512+Glutathione
S512
S512+Phage+Glutathione
S512+Phage
S512+Phage-Se+Glutathione
S512+Phage-Se



Survival Number of PYPR-1b Bacteria After Treatmen
with Phage-6 Labeled Selenium
400
350
300
250
200
150
100
50
0
0
24
Time in Hours


ER2738 Treated with Phage and Phage-Se with and without Glutathione
Number of Colonies
500
400
300
200
100
0
0
6
20
38
50
Time in hours
ER2738+Glutathione
ER2738
ER2738+Phage+Glutathione
ER738+Phage
ER2738+Phage-Se+glutathione
ER2738+Phage-Se

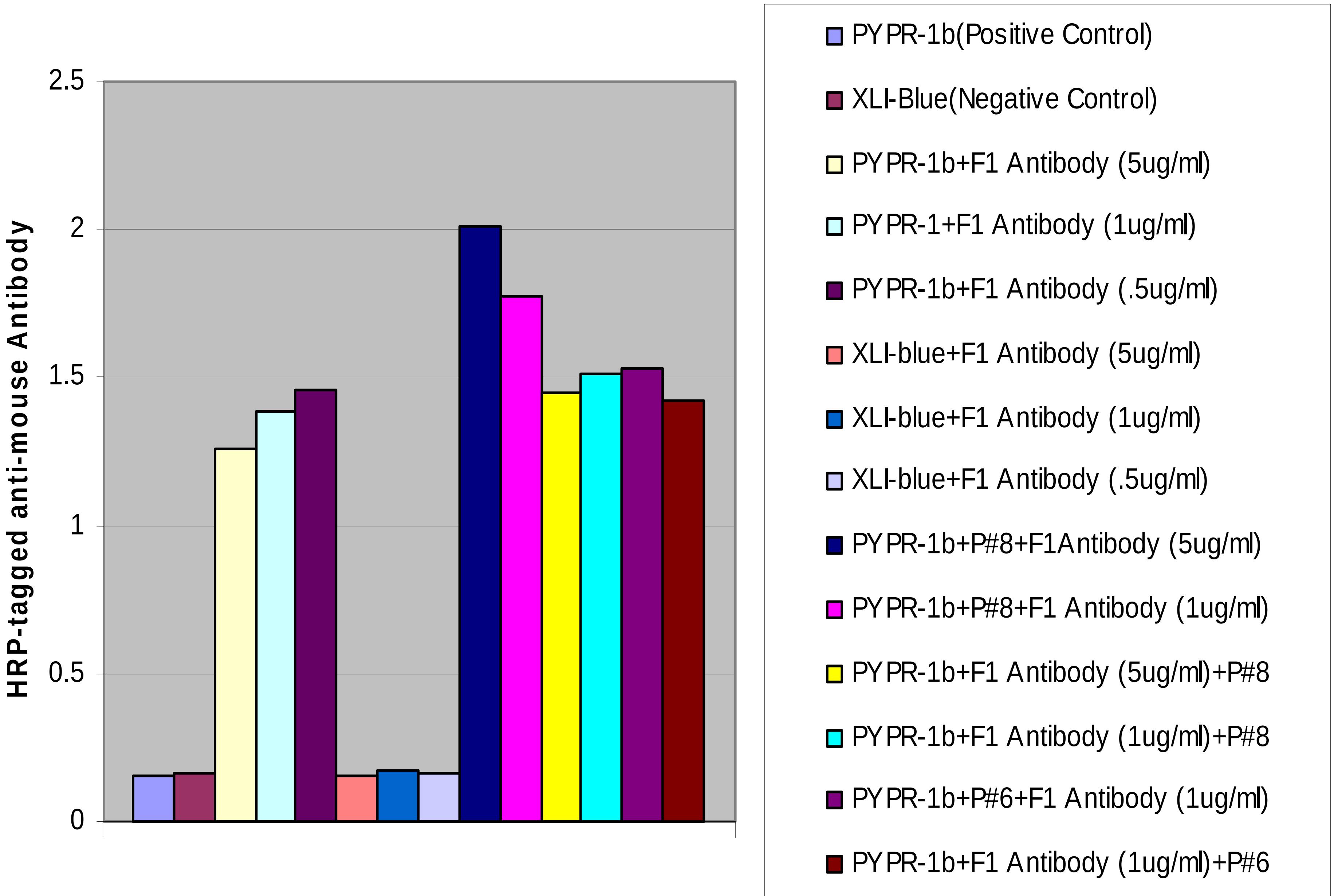


Kiling Curve Between Phage and Phage-Se
Blue Plaques
80
70
60
50
40
30
20
10
0
0
1
10
20
30
Time in Hours
Phage
Phage-Se

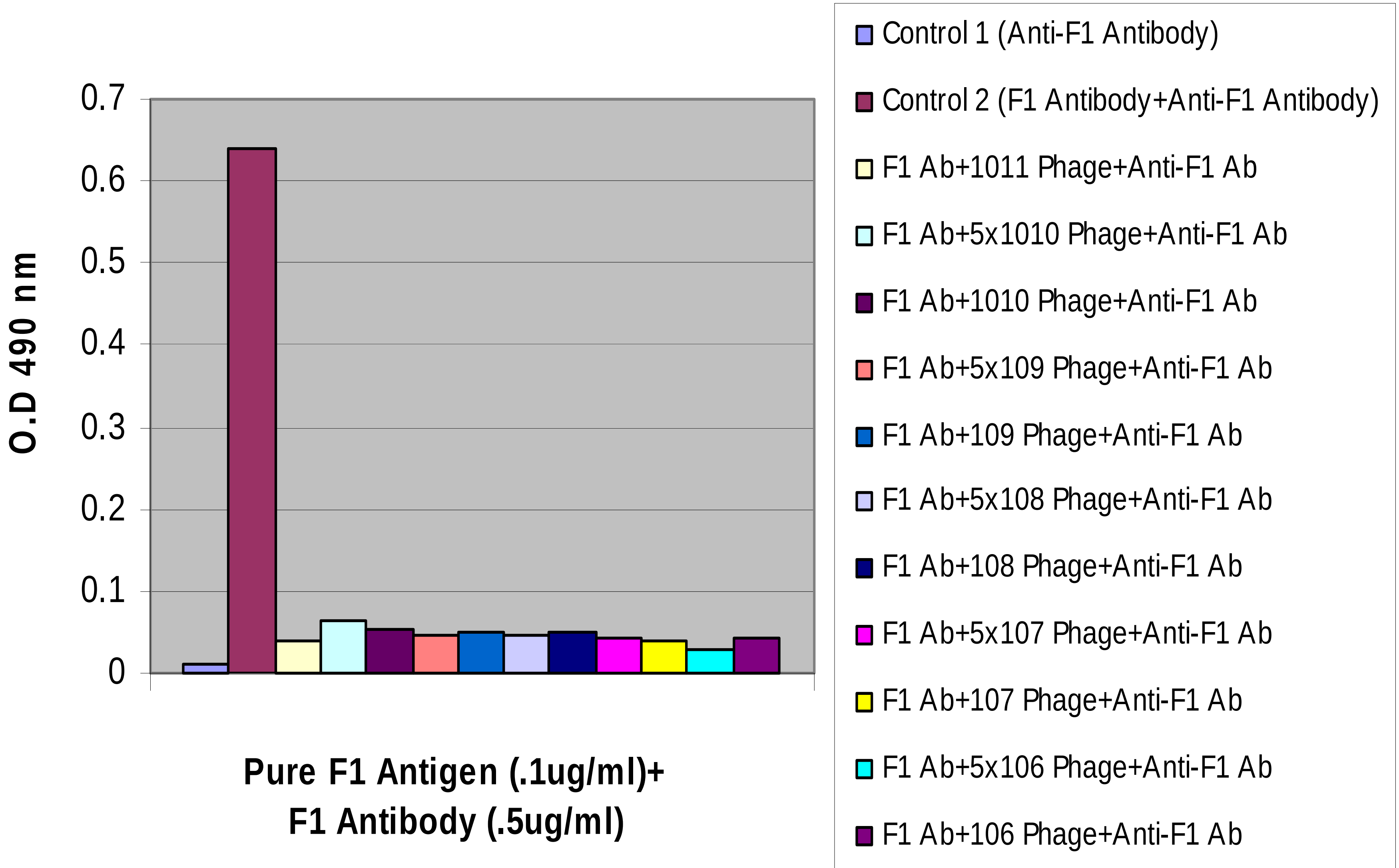
Killing curves for bacteria using selenium labeled phage with binding specificity for those bacteria

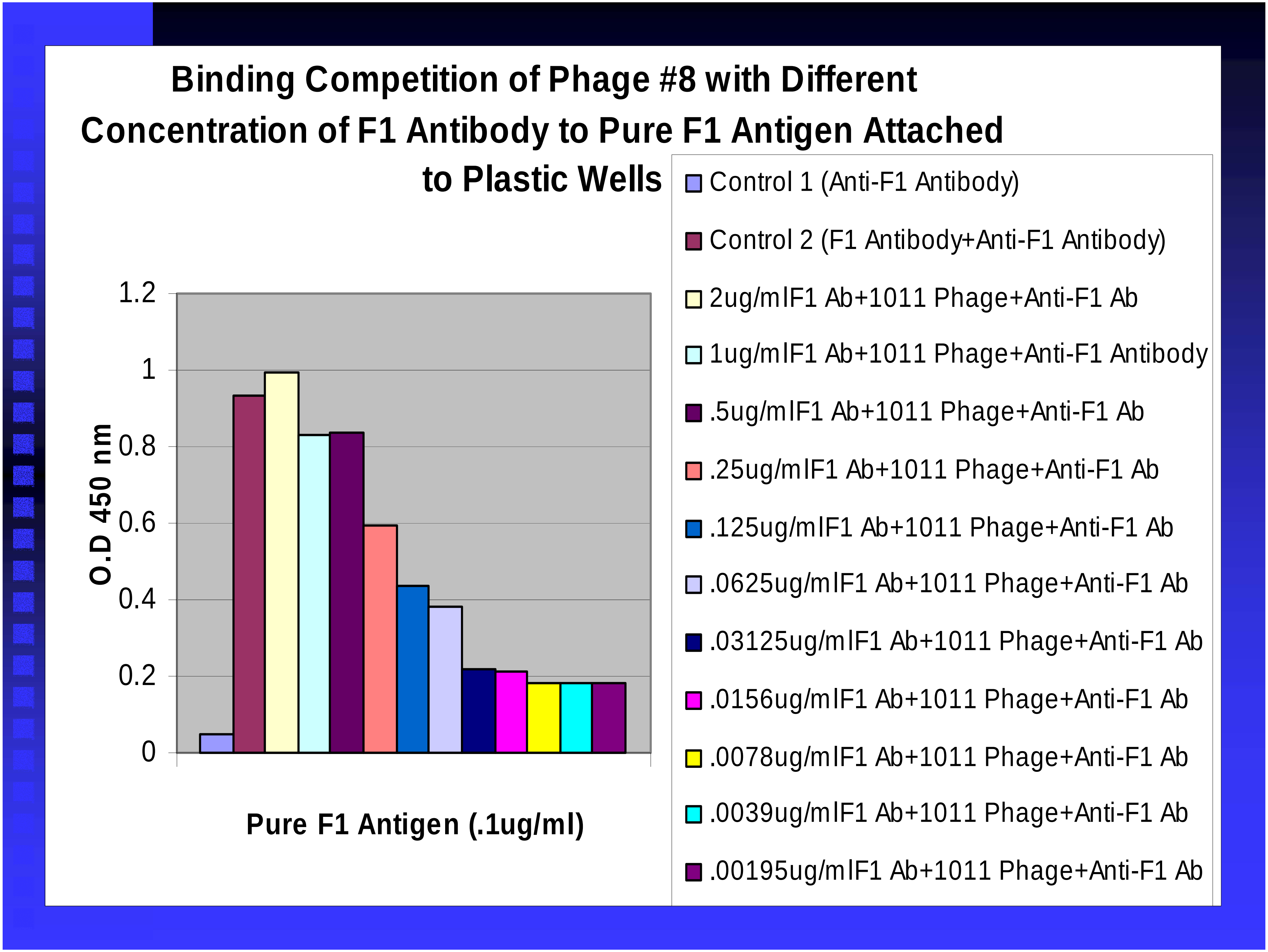
Phage mixed with bacteria for different periods of time under different reaction conditions. Bacteria then plated and counted.

Determine The Presence of F1 Antigen on The Surface of *E.coli* PYPR-1b Strain by ELISA Assay and The knock-out of F1 Antibody by Phage



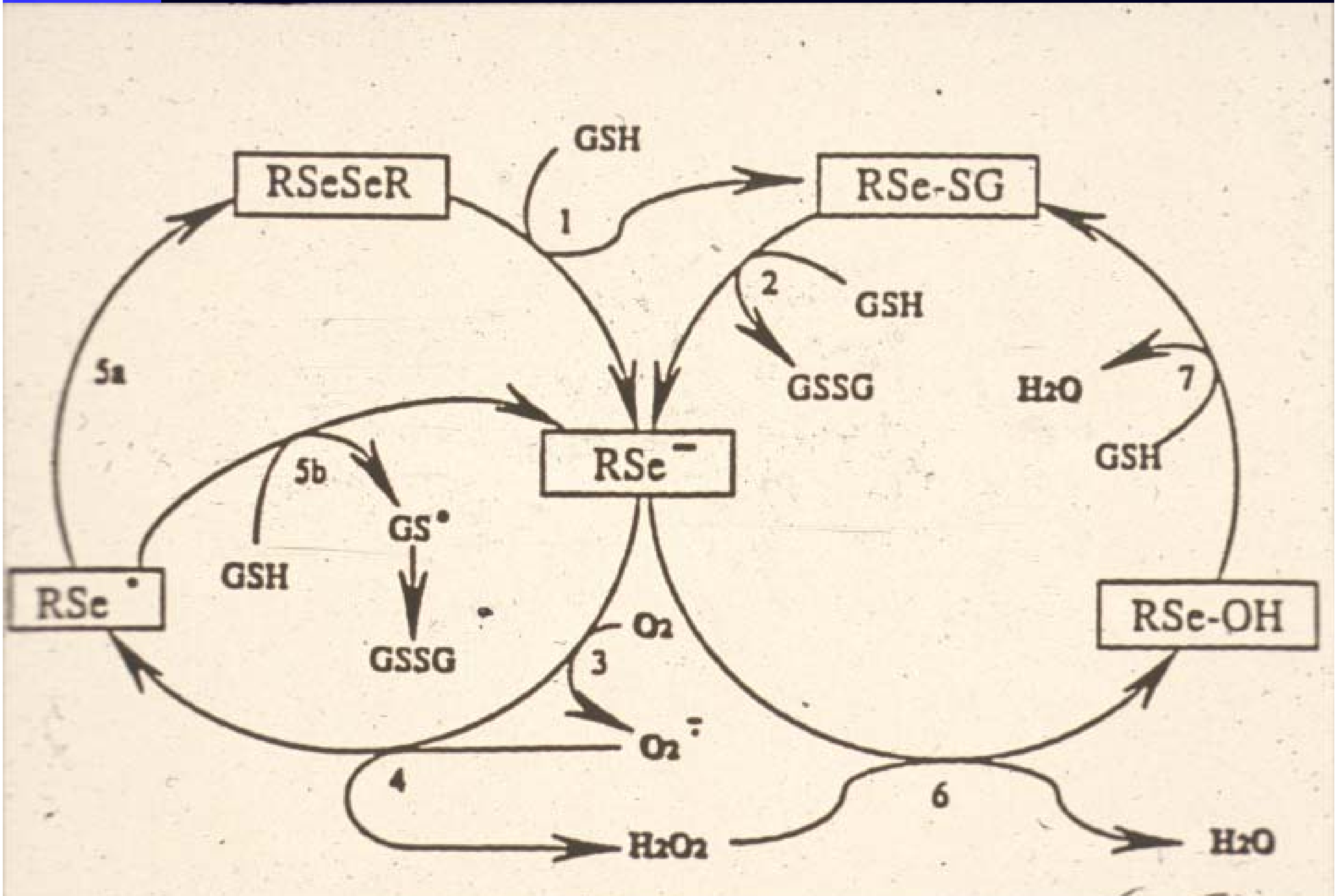
Binding Competition of Phage #8 with F1 Antibody to Pure F1 Antigen Attached to Plastic Wells




Binding Competition of Phage #8 with Different Concentration of F1 Antibody to Pure F1 Antigen Attached to Plastic Wells
O.D 450 nm
1.2
1
0.8
0.6
0.4
0.2
0
Pure F1 Antigen (.1ug/ml)
Control 1 (Anti-F1 Antibody)
Control 2 (F1 Antibody+Anti-F1 Antibody)
2ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
1ug/mlF1 Ab+1011 Phage+Anti-F1 Antibody
.5ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.25ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.125ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.0625ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.03125ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.0156ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.0078ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.0039ug/mlF1 Ab+1011 Phage+Anti-F1 Ab
.00195ug/mlF1 Ab+1011 Phage+Anti-F1 Ab

Conclusions

- Selenium can be attached to a bacterial virus which can still target and bind to the bacteria.
- A selenium labeled virus targeted for a specific bacteria can kill the bacteria.
- The killing of the bacteria is promoted by glutathione.



GSH
RSeSeR
1
RSe-SG
2
GSH
GSSG
5a
H2O
7
GSH
5b
RSe⁻
GS•
RSe•
GSH
GSSG
O2
3
RSe-OH
O2⁻
4
6
H2O2
H2O

QuickTime™ and a
GIF decompressor
are needed to see this picture.